(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 177 882**
B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: 03.01.90

(51) Int. Cl.[5]: C 07 G 11/00, C 12 P 1/06, A 61 K 35/70, C 12 R 1/03, C 07 K 15/02

(21) Application number: 85112406.5

(22) Date of filing: 01.10.85

(54) **Antibiotic A 40926 complex and its pure factors PA, PB, A, B and BO.**

(30) Priority: 11.10.84 GB 8425685

(43) Date of publication of application: 16.04.86 Bulletin 86/16

(45) Publication of the grant of the patent: 03.01.90 Bulletin 90/01

(84) Designated Contracting States: AT BE CH DE FR GB IT LI LU NL SE

(56) References cited: none

The file contains technical information submitted after the application was filed and not included in this specification

(73) Proprietor: GRUPPO LEPETIT S.P.A.
23, Via G. Murat
I-20159 Milano (IT)

(72) Inventor: Selva, Enrico
35, Via Carlo Cantoni
I-27027 Gropello Cairoli Pavia (IT)
Inventor: Gastaldo, Luciano
7 Via San Martino
I-20010 Pogliano Milanese Milan (IT)
Inventor: Beretta, Grazia
12, Via Belgirate
I-20125 Milano (IT)
Inventor: Borghi, Angelo
36, Via Perluigi da Palestrina
I-20124 Milano (IT)
Inventor: Goldstein, Beth P.
46, Corso Garibaldi
I-20121 Milano (IT)
Inventor: Arioli, Vittorio
9, Via Verdi
I-22070 Cassina Rizzardi Como (IT)
Inventor: Cassani, Giovanni
3, Via Vittadini
I-27100 Pavia (IT)
Inventor: Parenti, Francesco
8, Via Emilio De Marchi
I-20125 Milano (IT)

Courier Press, Leamington Spa, England.

EP 0 177 882 B1

(74) Representative: Sgarbi, Renato et al
GRUPPO LEPETIT S.p.A. Patent and Trademark Department 34, Via R bert Lepetit
I-21040 Ger nzan (Var se) (IT)

Description

The present invention concerns a new antibiotic substance arbitrarily denominated antibiotic A 40926 complex and its factors antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA and antibiotic A 40926 factor PB, a process for pr ducing them by culturing the new strain *Actinomadura sp.* ATCC 39727 or an A 40926 producing variant or mutant thereof and the use of these new antibiotic substances in the treatment of infectious diseases involving microorganisms susceptible to them.

The antibiotic substances of the invention are glycopeptidic substances belonging to the vancomycin class of antibiotics which have binding capacity to Acyl - D - Alanyl - D - Alanine.

Antibiotic A 40926 complex and its factors antibiotic A 40926 factor A, factor B, factor B_0, factor PA and factor PB, may form salts according to known *per se* techniques.

In the present description and claims the expression "antibiotic A 40926" *as such* represents a compound selected from antibiotic A 40926 complex, antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA, antibiotic A 40926 factor PB a salt thereof or any mixture thereof. The expressions "antibiotic A 40926 complex", "antibiotic A 40926 factor PA", "antibiotic A 40926 factor PB", "antibiotic A 40926 factor A", "antibiotic A 40926 factor B", and antibiotic A 40926 factor B_0, when dealing with the biological properties, will encompass also the corresponding pharmaceutically acceptable salts.

Antibiotic A 40926 is produced by cultivating an *Actinomadura* strain isolated from a soil sample and which has been deposited on June 8, 1984 at the internationally recognized collection American Type Culture Collection (ATCC)—12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty. The strain has been accorded the accession number ATCC 39727.

The producing strain was originally thought to belong to the genus *Streptomyces* and was initially denominated *Streptomyces nov. sp.* A 40926 and as such deposited with ATCC where it received the accession number ATCC 39727. Further studies, especially on cell-wall composition brought us to conclude that the new strain belongs to the genus *Actinomadura*. Accordingly, the strain originally deposited as *Streptomyces sp.* ATCC 39727 has been renamed *Actinomadura sp.* ATCC 39727.

The production of antibiotic A 40926 complex, antibiotic A 40926 factor A, factor B, factor B_0, factor PA, or factor PB, is achieved by cultivating an *Actinomadura sp.* capable of producing it, i.e. *Actinomadura sp.* ATCC 39727 or an antibiotic A 40926-producing variant or mutant thereof, under aerobic conditions in an aqueous nutrient medium containing assimilable sources of carbon, nitrogen, and inorganic salts. Many of the nutrient media usually employed in the fermentation art, can be used, however certain media are preferred. Preferred carbon sources are glucose, mannose, galactose, starch, corn meal and the like. Preferred nitrogen sources are ammonia, nitrates, soybean meal, peptone, meat extract, yeast extract, tryptone, aminoacids, and the like. Among the inorganic salts which can be incorporated in the culture media there are the customary soluble salts capable of yielding sodium, potassium, iron, zinc, cobalt, magnesium, calcium, ammonium, chloride, carbonate, sulfate, phosphate, nitrate and the like ions.

Ordinarily, the antibiotic-producing strain is pre-cultured in a shake flask, then the culture is used to inoculate jar fermentors for production of substantial quantities of the antibiotic substances. The medium used for the pre-culture can be the same as that employed for larger fermentations, but other media can also be employed. The antibiotic A 40926 producing-strain can be grown at temperatures between 20 and 40°C, preferably between 24 and 35°C.

During fermentation, the antibiotic production can be monitored by testing broth or mycelial extract samples for antibiotic activity for instance by bioassays or TLC or HPLC procedures.

Sensitive organisms to antibiotics A 40926 such as *Bacillus subtilis* and *S. aureus* can be used as test organisms. The bioassay is conveniently performed by the agar diffusion method on agar plates. Maximum production of antibiotic activity generally occurs between the second and the fifth day of fermentation.

Antibiotic A 40926 is produced by cultivating the strain *Actinomadura sp.* ATCC 39727, or an antibiotic A 40926 producing mutant or variant thereof, and is mainly found in the culture broths.

The characteristics of *Actinomadura sp.* A 40926 ATCC 39727 are given in the following paragraphs:

Macroscopic and microscopic examination

The vegetative mycelium is composed of flexuous and branched hyphae (about 0.8 µm of diameter) which on some media, identified by an asterisk in Table I, slightly tends to fragment into rod-like elements after several days of growth, while on glucose-asparagine medium it fragments into coccoid elements.

Characteristic of this strain is the Burgundy color of the vegetative mycelium on some media.

The aerial mycelium is present only in few media; in particular, among those listed in Table I, it is present only in oatmeal agar and soil agar. On these media the aerial mycelium is white-grey and forms sporophores arranged in h ks and sh rt spirals of about 10 to 20 spor s.

The spores are cylindrical and have an average size of 0.8×1.2 µm.

Determination of growth charact ristics

For the xamination f the cultural characteristics, *Actinomadura sp.* ATCC 39727 was cultivat d n

various standard media suggested by Shirling and Gottlieb (Shirling E. B. and Gottlieb D., 1966—Method for characterization of *Streptomyces* species—Int. J. Syst. Bacteriol, *16*, 313—340) with the addition of several media recomm nded by Waksman (Waksman, S. A. 1961—The Actinomycetes—The Williams and Wilkins Co. Baltimore; Vol. *2*, 328—334).

Color determinati n was made whenever necessary by the method of Maerz and Paul (Ma rz A. and M. Rea Paul, 1950—A Dictionary of Color—2nd Edition McGraw-Hill Book Company Inc. New York).

The ability of the organism to utilize different carbon sources was investigated by the method described by Shirling and Gottlieb.

The cultural and physiological characteristics and the carbon sources utilization are reported in Tables I, II, III.

The readings in Table I have been taken after two weeks incubation at 28°C.

TABLE I

Cultural characteristics of strain *Actinomadura sp* ATCC 39727

Culture media	Characteristics
Medium No. 2 (yeast extract—malt agar)	Abundant growth, with crusty surface, 8/L/8, traces of amber-pink soluble pigment
Medium No. 3 (oatmeal agar)	Abundant growth, with smooth surface, violet, 55/E/4, aerial mycelium very scant grey, soluble pigment violet 55/H/4
Medium No. 4 (inorganic salts—starch agar)	Moderate growth, with smooth and thin surface, cream 10/D/2
Medium No. 5 (glycerol—asparagine agar)	Moderate growth, with smooth and thin surface, apricot 10/B/2
Medium No. 6* (peptone—yeast extract iron agar)	Moderate growth, with slightly crusty surface, amber 12/D/9
Medium No. 7 (tyrosine agar)	Abundant growth, with smooth and thin surface amber-brown 13/K/12
Oatmeal agar*	Abundant growth, with smooth surface, Burgundy 8/L/7, aerial mycelium, moderate light yellow-grey 44/B/2
Hickey and Tresner's agar*	Abundant growth, with wrinkled surface, amber-brown 13/K/12
Czapeck glucose agar	Moderate growth, with smooth surface, light-yellow 9/I/3
Glucose asparagine agar*	Scant growth, with creamy surface, straw-yellow 9/E/1
Nutrient agar	Abundant growth, with wrinkled surface, light-orange 11/C/7
Potato agar*	Abundant gr wth, with wrinkled surface, Burgundy 8/L/9

TABLE I (cont.)
Cultural characteristics of strain *Actinomadura sp* ATCC 39727

Culture media	Characteristics
Bennett's agar*	Abundant growth, with crusty surface, Burgundy 8/L/8, soluble pigment deep amber rose 5/J/10
Calcium malate agar	Moderate growth, with smooth surface, apricot 10/B/3
Skim milk agar	Abundant growth, with slightly wrinkled surface, orange 9/B/9
Czapeck sucrose agar	Abundant growth, with smooth surface, apricot 10/B/6
Egg albumin agar*	Abundant growth, with smooth surface, rose 52/B/3, traces of a soluble pigment, rose 52/B/3
Sabouraud agar	No growth
Soil agar	Scant growth, colorless, white-grey aerial mycelium
Dextrose triptone agar*	Moderate growth, with smooth and thin surface, light-yellow 10/G/2
Potato plug	Abundant growth, orange-brown, traces of white-grey aerial mycelium

Physiological characteristics

TABLE II
Physiological characteristics

Tests		Results
Starch hydrolysis		negative
H_2S formation		negative on Medium No. 6 positive with lead acetate strips
Tyrosine reaction		positive
Casein hydrolysis		positive
Calcium malate hydrolysis		negative
Gelatin liquefaction		positive
Litmus milk	coagulation	negative
	peptonization	positive
Cellulose decomp sition		negative
Nitrate reducti n		positiv

Utilization of carbon sources

TABLE III
Carbon utilization

Carbon source	Growth
Arabinose	+
Xylose	+
Mannose	+
Fructose	+
Raffinose	+
Rhamnose	+
Glucose	+
Lactose	+
Galactose	+
Inositol	−
Sucrose	+
Cellulose	−
Salicin	+
Mannitol	+
Ribose	−

+=growth
−=no growth

Chemotaxonomical characteristics
Cell wall analysis:

The analysis of aminoacids present in the cell wall was carried out by the methods described in the work of Becker *et al.*, "Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole cell hydrolysates", Appl. Microbiol. *12*, 421—423 (1964). The analysis of the whole cell hydrolysate revealed the presence of *meso*-diaminopimelic acid.

The analysis of pure cell wall, obtained with the method of Kawamoto et al. (I. Kawamoto, T. Oka, and T. Nara, "Cell-wall composition of *Micromonospora olivoasterospora, Micromonospora sagamiensis*, and related organism", J. of Bacteriology *146*, 527—534, 1981) showed absence of glycine.

Sugar analysis:

The analysis of sugar content was carried out by the method of M. P. Lechevalier, "Identification of aerobic actinomycetes of clinical importance", J. Lab. Clin. Med. *71*, 934—944 (1968) using thin layer chromatography cellulose sheets as described by J. L. Staneck and G. D. Roberts, "Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography", *28*, 226—231 (1974) with the following solvent system: Ethylacetate-Pyridine-Water (100:35:25 by volume). The obtained results showed the presence of mainly glucose and ribose while lower quantities of galactose, mannose and madurose (3-O-methyl-D-galactose) were also detected.

Mycolic acids:

An assay for detecting the presence of mycolic acids was carried out by the following method of Minnikin *et al.* (D. E. Minnikin, L. Alshamaony and M. Goodfellow, "Differentiation of *Mycobacterium, Nocardia* and related taxa by thin layer chromatography analysis of whole organism methanolysates", Journal of General Microbiology *88*, 200—204, 1975):

The results of the assay were negative: mycolic acids were not found.

Identity of the strain:

The strain is assigned to the Actinomycetes genus *Actinomadura* because of the presence of meso-diaminopimelic acid and madurose, the lack of glycine in the peptodoglycan, the lack of mycolic acids and the formation of aerial mycelium with moderately long spore chains.

As with other microorganisms, the characteristics of the A 40926 producing strain are subject to variation. For example, artificial variants and mutants of the strain can be obtained by treatment with various known mutagens, such as U.V. rays, X-rays, high frequency waves, radioactive rays, and chemicals such as nitrous acid, N - methyl - N' - nitro - N - nitrosoguanidine, and many others. All natural and artificial variants and mutants which belong to the species of the genus *Actinomadura* and produce A 40926 antibiotics, are deemed *equivalent* to strain *Actinomadura sp.* ATCC 39727 and are contemplated to be within the scope f this inventi n.

The rec v ry of the antibiotic substances of th inv nti n from the fermentation broths f the producing microorganism is c nducted according to kn wn *per se* techniques which include extraction with solvents, precipitation by adding non-solvents or by changing the pH of the solution, partition

chromatography, reverse-phase partition chromatography, ion-exchange chromatography, affinity chromatography and the like.

A preferred procedure includes an affinity chromatography on immobilized D - Alanyl - D - Alanine followed by reverse-phase column chromatography.

Immobilized D-Alanyl-D-Alanine matrices suitable for the present recovery process are disclosed in European Patent Application No. 83112555. The preferred matrix in the present process is D-Alanyl-D-Alanine coupled with a controlled pore cross-linked polydextrane.

The fermentation broth can be subjected to the affinity chromatography directly after filtration or after a preliminary purification procedure. This latter procedure includes making the whole fermentation mass basic, preferably between pH 8.5 and 10.5, in order to solubilize the antibiotic substance adsorbed on the mycelium and then filtering. The clear filtrate is brought to pH 2.5—4.5 and filtered again in the presence of a filter aid. This filtrate is discarded while the recovered filtration cake is suspended in water, made basic, preferably at a pH between 8 and 9, and filtered. The filtration cake is re-subjected to the same procedure while the filtrates, which contain antibiotic A 40926, are pooled.

These filtrates or the filtered fermentation broths are then subjected to an affinity chromatography on immobilized D-Alanyl-D-Alanine, either in column or batchwise.

The binding of the antibiotic substance to the affinity matrix is preferably made at a pH of about 7.0—8.0 and its elution is performed at more basic pH values (preferably between 9.0 and 10.5) by means of an aqueous base. This aqueous base may be ammonia, a volatile amine, an alkali metal or alkaline-earth metal hydroxide or a basic buffered solution optionally in the presence of a polar organic solvent such as a polar water-miscible solvent as defined below.

After removing the impurities by rinsing the column with aqueous buffer pH 4—8, optionally containing salts, urea and/or water miscible solvents, the antibiotic A 40926 is eluted with the above eluting mixture. The crude antibiotic substance is then recovered preferably by removing water from the pooled antibiotic-containing fractions by azeotropical distillation with an organic solvent capable of forming minimum azeotropic mixtures with water, followed by addition of a non-solvent to precipitate the desired product.

Representative examples of organic solvents capable of forming minimum azeotropic mixtures with water are n-butanol, benzene, toluene, butyl ether, carbon tetrachloride, chloroform, cyclohexane, 2,5 - dimethylfurane, hexane and m-xylene; the preferred solvent being n-butanol.

Examples of non-solvents are: petroleum ether, lower alkyl ethers, such as ethyl ether, propyl ether and butyl ether, and lower alkyl ketones such as acetone. Alternatively, the pooled antibiotic-containing fractions are concentrated to a small volume, preferably by azeotropical distillation with an organic solvent defined as above, and the resulting aqueous solution is lyophilized.

If the aqueous base employed in the elution is unvolatile, it may be necessary to neutralize and desalt the concentrate before precipitation or freeze-drying.

A convenient desalting procedure includes applying the antibiotic containing aqueous solution to a silanized silica gel column, washing with distilled water and eluting with a mixture of a polar water-miscible solvent and water.

Representative examples of polar water-miscible solvents are: water-soluble alcohols, (such as methanol, ethanol, iso-propanol, n-butanol), acetone, acetonitrile, lower alkyl alkanoates (such as ethyl acetate), tetrahydrofuran, dioxane and dimethylformamide and mixtures thereof; the preferred polar water-miscible solvent being acetonitrile.

Alternatively, desalting may be carried out by applying the antibiotic containing solution to the above described affinity column, washing with distilled water and eluting with a volatile aqueous base as described above for the elution of the affinity chromatography. The product so obtained is antibiotic A 40926 complex. If necessary, it may be further purified or subjected as such to the separation of its factors A, B, B_0, PA and PB.

A convenient procedure to obtain a pure antibiotic A 40926 complex is represented by a further purification of the complex as obtained above on an affinity chromatography column. The same stationary phase as above (immobilized D-Alanyl-D-Alanine) is generally used and the desired antibiotic substance is eluted by following the affinity chromatography procedure on immobilized D-Alanyl-D-Alanine described above. A preferred immobilized D-Alanyl-D-Alanine is Sepharose - ε - aminocaproyl - D - Alanyl - D - Alanine, a preferred equilibrating mixture is 0.16% (w/v) ammonia containing 2M NaCl adjusted to pH 7—8, a preferred rinsing solution is 0.16% (w/v) ammonia containing 2M NaCl adjusted to pH 8—9.5, a preferred eluting mixture is 0.16% (w/v) ammonia containing 2M NaCl adjusted to pH 10.5—12 and a most preferred eluting mixture is the above mixture adjusted to pH 11.5.

The antibiotic A 40926 factors, namely antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA and antibiotic A 40926 factor PB are isolated from an aqueous solution of antibiotic A 40926 complex by column chromatography and preferably by rev rse-phase column chromatography. The pref rred stati nary phase in the case of reverse-phase column chr matography is silanized silica g l. Go d results may be obtained however also with c lumn chromatography on n n-functi nalized polystyrene and acrylic resins such as those sold under the trade names Amberlite XAD-2, XAD-4, XAD-7 and XAD-8 (Rohm and Haas) or Diaion HP 20 (Mitsubishi).

In case the r v rse-phase purification st p is accomplished by means of a silanized silica g l as the

stationary phase, the column is preferably pre-equilibrated with a buffered aqueous solution at pH between 4 and 9 and preferably between 5.5—6.5 and then eluted with a linear gradient of a polar water-miscible solvent in the same buffered solution. Representative examples of polar water-miscible solvents are: water-soluble alcohols, (such as methanol, ethanol, iso-propanol, n-butanol), acetone, acetonitrile, tetrahydrofuran, dioxane and dimethylformamide and mixtures thereof; the preferred polar water-miscible solvent being acetonitrile.

The eluted fractions are assayed for their antibiotic content by means of the usual bioassays, such as paper-disc or agar-diffusion assays, on susceptible microorganisms. Examples of susceptible organisms are *Bacillus subtilis* and *S. aureus*.

The chromatography is also conveniently monitored by TLC or HPLC techniques.

A preferred HPLC technique is represented by a reverse-phase HPLC using a column of porous and spheric particles of silanized silica gel functionalized with C-18 alkyl groups having a diameter preferably of 5 micrometers (such as 5 µm Ultrasphere® ODS Altex; Beckman Co.), a pre-column which is a silica gel functionalized with C-18 alkyl groups (such as RP 18 Brownlee Labs) and an eluent which is a linear gradient mixture of a polar water miscible solvent, such as one of those described above, in an aqueous buffered solution.

Preferably this solution is adjusted to pH 5—7. A most preferred eluent is represented by a linear gradient from 5 to 60% of element B in eluent A wherein eluent A is a mixture of acetonitrile/aqueous buffer, pH 5—7, 10:90 and eluent B is a mixture of acetonitrile/aqueous buffer, pH 5—7, 70:30. As known in the art, many substances can be used as internal standards. A very convenient one is, in this case, Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.) which has a retention time close to the compounds of the invention in this HPLC system. This standard substance is known and has been described in GB—A—2121401.

Fractions with a similar antibiotic content are pooled and desalted as described above to give essentially pure antibiotic A 40926 factor A, factor B, factor B_0, factor PA, and factor PB.

Essentially pure antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA and antibiotic A 40926 factor PB are obtained from those fractions containing them by a variety of known techniques such as lyophilization, precipitation by non-solvents or precipitation by changing the pH of the aqueous solution.

A convenient procedure includes adding a solvent capable of forming azeotropic mixtures with water, removing water by azeotropic distillation and then collecting by filtration the precipitate obtained after addition of a non-solvent like those described above.

Antibiotic A 40926 factors PA and PB, at least under certain fermentation conditions, are the main antibiotic products of the A 40926 producing microorganism. Antibiotic A 40926 factors A and B are mainly transformation products of antibiotic A 40926 factor PA and factor PB respectively, and are often already present in the fermentation broth.

It has been found in fact that antibiotic A 40926 factor PA can be transformed into antibiotic A 40926 factor A and antibiotic A 40926 factor PB can be transformed into antibiotic A 40926 factor B under basic conditions. For instance, antibiotic A 40926 factor PA and antibiotic A 40926 factor PB are transformed into antibiotic A 40926 factor A and factor B respectively, by treatment with 0.5—10% aqueous ammonia or other nucleophilic base such as an organic amine at room temperature for 8—24 hours.

As a consequence, when the fermentation broth, or an antibiotic A 40926 containing extract or concentrate thereof, is allowed to stand for a certain time under basic conditions (e.g. aqueous solution of a nucleophilic base, at a pH >9 overnight), an antibiotic A 40926 complex will be obtained which is enriched in antibiotic A 40926 factor A and factor B. If the period of exposure of the fermentation broth, extract or concentrate thereof, to a basic environment is short, an antibiotic A 40926 complex is obtained which is enriched in antibiotic A 40926 factor PA and factor PB.

A preferred procedure to obtain an antibiotic A 40926 complex enriched in factor A and factor B includes therefore leaving a solution of antibiotic A 40926 complex (which contains mainly antibiotic A 40926 factors PA and PB) at room temperature for 8—12 h in an aqueous nucleophilic base, such as aqueous ammonia, and then isolating the desired antibiotic complex as described above.

Examples of A 40926 containing solutions are fermentation broths, extracts and affinity chromatography eluted fractions.

Pure antibiotic A 40926 may be obtained by further purifying the crude complex by affinity chromatography as described above.

The product so obtained which possesses biological and physico-chemical properties derivable from the pure factors thereof, will be referred to in the examples as antibiotic A 40926 complex AB.

A preferred procedure to enrich in factors PA and PB an antibiotic A 40926 complex preparation, includes rapidly neutralizing the affinity chromatography eluted fractions with an acid, preferably a mineral acid such as sulfuric or hydrochloric acid.

The isolation f pure antibiotic A 40926 factors PA and PB from this complex can be achieved according to one of the above reported pr cedures.

A preferred procedur includes r verse-phase liquid chromat graphy, preferably in stainless st l columns und r moderat pressur (5—50 bar) or at high pressur (100—200 bar). The solid phase may be a silanized silica gel with a hydrocarbon phase at (2—18) carbon atoms (m st preferably C 18) or phenyl

group, and the eluent is a mixture of a polar water-miscible solvent as defined above and an aqueous buffer at a pH compatible with the resin (preferably pH 4—8).

The elution is monitored as usual, the fractions having homogeneous antibiotic content are pooled and treated as described above to isolate the pure compounds having the characteristics reported below.

Sophisticated HPLC analysis has shown that antibiotic A 40926 factor B actually is a mixture of two factors denominated factor B_0 and factor B_1.

Antibiotic A 40926 factor B_0, which accounts for approximately 90% of antibiotic A 40926 factor B, is the factor that has R_t of 1.22 relative to Teicoplanin A_2 component 2 in the system described below under point D of the physico-chemical characteristics of factor B, while factor B_1, which accounts for approximately 10% of antibiotic A 40926 factor B, is that with relative R_t of 1.27 in the same system.

Pure antibiotic A 40926 factor B_0 is obtained by further purification of antibiotic A 40926 factor B for instance by repeating the reverse-phase chromatography procedure used for its isolation.

The physico-chemical and biological properties of antibiotic A 40926 factor B_0 are substantially identical to those of antibiotic A 40926 factor B except that at the HPLC analysis in a system like the above cited one, it has only one peak (R_t 1.22 relative to Teicoplanin A_2 component 2 in the described HPLC system).

Because of the above outlined similarities between antibiotic A 40926 factor B and antibiotic A 40926 factor B_0 in the present disclosure and claims the reference to the biological properties of antibiotic A 40926 factor B is to be understood as referring also to antibiotic A 40926 factor B_0 which is the main component (about 90%) of antibiotic A 40926 factor B and mainly contributes to its biological properties.

Alternatively, the antibiotic substances of the invention may be isolated from the fermentation broth or further purified by means of strong or weak anion resins including functionalized polystyrene, acyrlic or polydextrane matrices. Examples of weak anion exchange resins are those sold under the following trade-names: Dowex MWA-1 or WGR (Dow Chemical), Amberlite IRA-73 (Rohm and Haas), DEAE-Sephadex (Pharmacia). Examples of strong anion exchange resins which may be used according to invention include those sold under the following trade names: Dowex® MSA-1, SBR, SBR-P® (Dow Chemical), Amberlite IR-904® (Rohm and Haas) and QAE-Sephadex® (Pharmacia).

The elution of the antibiotic substances of the invention from these resins is conducted by means of linear gradient mixtures of aqueous solution of electrolytes, such as sodium or potassium hydrochlorides, in water or mixtures of water and an organic water-miscible solvent such as a lower alcohol (e.g. (C_1—C_4) alkanol) or lower alkyl ketones (e.g. acetone).

As already said, the antibiotic substances of the invention possess acid and basic functions and can form salts according to conventional procedures. Representative and suitable acid addition salts of the compounds of the invention include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoro-acetic, trichloroacetic, succinic, citric, ascorbic, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic and the like acids.

Representative examples of these bases are: alkali metal or alkaline-earth metal hydroxide such as sodium, potassium, and calcium, hydroxide; ammonia and organic aliphatic, alicyclic or aromatic amines such as methylamine, dimethylamine, trimethylamine, and picoline.

The transformation of the free amino or non-salt compounds of the invention into the corresponding addition salts, and the reverse, i.e. the transformation of an addition salt of a compound of the invention into the non-salt or free amino form, are within the ordinary technical skill and are encompassed by the present invention.

For instance, a compound of the invention can be transformed into the corresponding acid or base addition-salt by dissolving the non-salt form in an aqueous solvent and adding a slight molar excess of the selected acid or base. The resulting solution or suspension is then lyophilized to recover the desired salt.

In case the final salt is unsoluble in a solvent where the non-salt form is soluble it is recovered by filtration from the organic solution of the non-salt form after addition of the stoichiometric amount or a slight molar excess of the selected acid or base. The non-salt form can be prepared from a corresponding acid or base salt dissolved in an aqueous solvent which is then neutralized to free the non-salt form.

When following the neutralization desalting is necessary, a common desalting procedure may be employed. For example, column chromatography on silanized silica gel, non-functionalized polystyrene, acrylic and controlled pore polydextrane resins (such as Sephadex LH 20) or activated carbon may be conveniently used. After eluting the undesired salts with an aqueous solution, the desired product is eluted by means of a linear gradient or a step-gradient of a mixture of water and a polar or apolar organic solvent, such as acetonitrile/water from 50:50 to about 100% acetonitrile.

As it is known in the art, the salt formation either with pharmaceutically acceptcable acids (bases) or non-pharmaceutically acceptable acids (bases) may be used as a convenient purification technique. After formation and is lation, the salt form of an A 40926 antibiotic can be transformed into the corresponding non-salt or into a pharmaceutically acc ptable salt.

In some instances, the base addition salt of a compound of the invention is mor soluble in water and hydrophilic s lvents.

Physico-chemical characteristics of antibiotic A 40926 factor A

A) ultraviolet absorption spectrum, which is shown in Figure 1 of the accompanying drawings, and exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	281
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) methanol	282
e) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which is shown in Figure 2 of the accompanying drawings and exhibits the following absorption maxima (cm^{-1}):

3700—3100, 3100—2800 (nujol); 1655; 1620—1560; 1510; 1480—1410 (nujol); 1375 (nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (nujol).

C) ^{1}H-NMR spectrum which is shown in Figure 3 and exhibits the following groups of signals (in ppm) at 270 MHz recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

δ0.86 (t's, 6H); 1.21 (~11H); 1.43 (2H); 2.01 (2H); 2.31—2.34 (3H); 4—6.2 (~16H); 6.2—8 (~23H); 8.44, 9.22, 9.66 (broad bands; mobile protons)

2.5—4: interference from H_2O peaks.

D) retention-time (R_t of 0.60 relative to Testosterone when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere ODS (5 µm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Testosterone (Roussel Uclaf)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 4.6%) which indicates the following approximate percentage composition (average): carbon 55.82%; hydrogen 5.17%; nitrogen 6.31%; chlorine (total) 4.24%; chlorine (i nic) 0.37%. Inorganic residue at 900°C in the air: 1.2%.

F) acid-base titration profile in 2-methoxyethan l (MCS):water, 4:1 up n titration with KOH after addition of an excess of HCl which indicat s four ionizable functions having the following pk_{MCS}: 4.6, 5.6, 7.2, 9.2.

G) R_f value of 0.24 and a R_f value relative to Teicoplanin A_2 component 2 of 0.70 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1716 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1717.

Physico-chemical characteristics of antibiotic A 40926 factor B

A) ultraviolet absorption spectrum, which is shown in Figure 4 of the accompanying drawings, and exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) infrared absorption spectrum which is shown in Figure 5 of the accompanying drawings and exhibits the following absorption maxima (cm^{-1}):
3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^{1}H-NMR spectrum shown in Figure 6 exhibits the following groups of signals (in ppm) in the 270 MHz ^{1}H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):
δ 0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons) 2.5—4 interference from H_2O peaks.

D) Retention times (R_t) of 1.22 and 1.27 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient fr m 5% to 60% of elu nt B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 9.6%) indicates the following approximate percentage composition (average): carbon 54.09%; hydrogen 5.13%; nitrogen 6.34%; chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl (pH 2.7) which indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the M+H[⊕] peak at 1731.

Physico-chemical characteristics of antibiotic A 40926 factor B_0

A) ultraviolet absorption spectrum which is shown in Figure 4 of the accompanying drawings and exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) infrared absorption spectrum which shown in Figure 5 of the accompanying drawings exhibits the following absorption maxima (cm^{-1}):
3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^{1}H-NMR spectrum which shown in Figure 6 exhibits the following groups of signals (in ppm) in the 270 MHz ^{1}H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):
δ0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons)
2.5—4 interference from H_2O peaks.

D) Retention time (R_t) of 1.22 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-c lumn:
Brownlee® Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 9.6%) indicates the following approximate percentage composition (average):

carbon 54.09%; hydrogen 5.13%; nitrogen 6.34%; chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1731.

Physico-chemical characteristics of antibiotic A 40926 factor PA

A) ultraviolet absorption spectrum, shown in Figure 7 of the accompanying drawings, exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	283 300 (shoulder)

B) infrared absorption spectrum shown in Figure 8 of the accompanying drawings exhibts the following absorption maxima (cm^{-1}):

3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (nujol); 1375 (nujol); 1260, 1250—950; 845; 805; 720 (nujol)

C) ^1H-NMR spectrum which is shown in Figure 9 exhibits the following groups of signals (in ppm) in the 270 MHz ^1H-NMR record d in DMSO d_6 (h xadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

0.86, d's (CH_3); 1.15—1.22, m $(CH_2)_n$; 1.41, m (CH_2); 2.01, s (CH_3); 2.01, m (CH_2); 2.28, s (N-CH_3); 4.26—5.96, br (peptidic and aromatic CH's); 6.33—7.73 br (aromatic CH's and peptidic NH's).

br = broad
d = doublet
dd = doublet of doublets
m = multiplet
s = singlet
t = triplet

D) retention-time (R_t) of 1.15 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.62 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

F) MW of about 1758 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1761. The operative conditions of the FAB-MS analysis were the following:

Instrument:
VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:
Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol - glycerol 1/1 (v/v).

Physico-chemical charact ristics f antibiotic A 40926 factor PB

A) ultraviolet absorption spectrum, sh wn in Figure 10 of the accompanying drawings, exhibits the f ll wing abs rpti n maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which shown in Figure 11 of the accompanying drawings exhibits the following absorption maxima (cm^{-1}):
3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (nujol); 1375 (nujol); 1320—1270; 1230—1190; 1150, 1120—920; 845; 810; 720 (nujol)

C.1) ^{1}H-NMR spectrum shown in Figure 12 exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):
0.84, d (isopropyl CH_3's); 1.17, m $(CH_2)_n$; 1.43, m (CH_2), 1.99, s (CH_3); 2.01, m (CH_2); 2.31, s $(N\text{-}CH_3)$; 2.79, dd (C-H); 3.70, m (C-H); 4.06—6.02, br (peptidic and aromatic CH's); 6.45—7.74, br (aromatic CH's and peptidic NH's); 8.19—9.99, br (peptidic NH's and phenolic OH's)
C.2) ^{1}H-NMR spectrum shown in Figure 13 exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 plus CF_3COOD using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):
0.84, d (isopropyl CH_3's); 1.13, m $(CH_2)_n$; 1.40, m (CH_2); 1.98, s (CH_3); 2.00, m (CH_2); 2.92, dd (C-H); 3.29—3.71, m (sugar C-H's); 4.07—6.09, s and m (peptidic and aromatic CH's); 6.45—7.83, s and m (aromatic CH's and peptidic NH's); 8.17—10.38 (peptidic NH's, phenolic OH's).

D) retention times (R_t) of 1.27 and 1.32 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 μm) Altex (Beckman 4.6 mm (I.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqu ous Na_2SO_4	70
acet nitrile	30

using silaniz d silica gel 60 F_{254} Merck plates (layer thickn ss 0.25 mm)

Visualization:

—U.V. light

—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))

—Bioautography using *B. subtilis* ATCC 6633 n minimal Davis medium.

F) MW of about 1772 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1776. The operative conditions of the FAB-MS analysis were the following:

Instrument:

VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:

Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol-glycerol 1/1 (v/v).

The antibacterial activity of the compounds of the invention can be demonstrated *in vitro* by means of standard dilution tests on different microorganism cultures.

Culture media and growth conditions for MIC (minimal inhibitory concentration) determinations were as follows: Isosensitest broth (Oxoid), 24 h, for staphylococci, *Strep. faecalis* and Gram-negative bacteria (*Escherichia coli, Pseudomonas, Proteus*); Todd-Hewitt broth (Difco), 24 h for other streptococcal species; GC base broth (Difco)+1% Isovitalex (BBL), 48 h, CO_2-enriched atmosphere for *Neisseria gonorrhoeae*; GB base agar (Difco)+1% Isovitalex+0.001% hemin, 24 h for *Haemophilus influenzae;* AC broth (Difco), 24 h, anaerobic atmosphere for *Clostridium perfringens;* Wilkins-Chalgren agar (ref: T. D. Wilkins & S. Chalgren, 1976, Antimicrob. Ag. Chemother. *10,* 926), 48 h, anaerobic atmosphere for the other anaerobes (*C. difficile, Propionibacterium acnes, Bacteriodes fragilis*); PPLO broth (Difco)+10% horse serum+1% glucose, 48 h for *Mycoplasma gallisepticum;* Yeast nitrogen base broth (Difco), 24 h for *Candida albicans.* With the exception of *C. albicans* (30°C), incubation was at 37°C. Inocula were as follows: 1% (v/v) of a 48 h broth culture for *M. gallisepticum;* about 10^4—10^5 colony-forming units/ml for other broth dilution MICs; about 10^4—10^5 bacteria/spot (inoculated with a multipoint inoculator) for agar dilution MICs (*H. influenzae, C. difficile, P. acnes, B. fragilis).*

TABLE IV

Strain	Antibiotic A 40926 Factor A	Factor B	Factor PA	Factor PB
	M.I.C. (µg/ml)			
Staph. aureus ATCC 6538	0.13	0.13		
Staph. aureus Tour (L165) 10^4 cfu/ml	0.13	0.13	0.5	1
Staph. aureus Tour (L165) 10^6 cfu/ml	0.25	0.13	2	2
Staph. epidermidis ATCC 12228			4	4
Strept. mitis L 796 (clin. is.)			0.06	0.06
Strep. pyogenes C203	0.063	0.063	0.06	0.03
Strep. pneumoniae UC41	0.063	0.063	0.06	0.06
Strep. faecalis ATCC 7080	0.13	0.13	0.06	0.13
Clostr. perfringens ISS 30543	0.016	0.016	0.008	0.008
Clostr. difficile ATCC 9689	0.25	0.13	0.25	0.13
Propion. acne ATCC 6919	0.03	0.016	0.03	0.03
Neisseria gonorrhoeae L 997 (clin. isol.)	1	0.5	4	2
Haemophilus influenzae ATCC 19418	64	64	64	64
Proteus vulgaris X19H ATCC 881	>128	>128	>128	>128
Escherichia coli SKF 12140	>128	>128	>128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	>128
Bacteroides fragilis ATCC 23745	64	64	64	64
Candida albicans SKF 2270	>128	>128		
Mycoplasma gallisepticum Weybridge	64	64	128	64

TABLE V
Bactericidal effect on *N. gonorrhoeae* strains

N. gonorrhoeae strain	Antibiotic A 40926 complex MIC (48 h)	Antibiotic A 40926 complex MBC (24 h)	Spectinomycin MIC (48 h)	Spectinomycin MBC (24 h)
L 1001*	1	1	16	32
L 1004*	1	0.5	16	32
L 1007 (CTC 6820)	2	2	16	16
L 1596**	2	2	>128	>128
L 1601***	1	1	16	16
L 1605***	2	2	32	32

MIC=minimal inhibitory concentration
MBC=minimal bactericidal concentration (lowest concentration at which 99.9% of the initial inoculum is killed within the indicated time)
*=clinical isolate
**=clinical isolate, spectinomycin-resistant
***=clinical isolate, penicillin-resistant

The antimicrobial activity of the compounds of the invention is confirmed also in *in vivo* experiments conducted essentially as described by R. Pallanza et al., J. Antimicrob. Chemother. *11*, 419 (1983).

The experimental infection was induced in mice by intraperitoneally administering a suspension of *S. pyogenes* C 203. Inocula had been adjusted so that the untreated animals died of septicemia within 48 h. Animals were treated subcutaneously with the test compound about 30 min after infection.

The ED_{50} value was calculated on the 10[th] day by the method of Spearman and Karber (D. J: Finney "Statistical Methods in Biological Assay", Griffin, page 524, 1952) on the basis of the percentage of survival at each dose. In the above conditions the ED_{50} for antibiotic A 40926 factor A was 0.47 mg/kg, for antibiotic A 40926 factor B was 0.33 mg/kg, for antibiotic A 40926 factor PA was 0.54 mg/kg and for antibiotic A 40926 factor PB 0.31 mg/kg.

The approximate acute toxicity in mice (i.p.) was evaluated according to methods known in the art. The approximate LD_{50} of antibiotic A 40926 was found to be higher than 100 mg/kg in mice, when administered subcutaneously.

Antibiotic A 40926 complex and its factors A, B, B_0, PA and PB are active against gram-positive bacteria which are responsible for many widely diffused infections. Because of the increasing resistance of these pathogens to the usual therapeutic treatments, the need for new antibiotic substances is still great.

As already stated, the antibiotic substances of the invention possess a good activity against *Neisseria* strains, such as *Neisseria gonorrhoeae*, while they are practically inactive against other gram-negative bacteria.

It is known that the incidence of gonorrhea has risen steadily in the last 15—20 years.

The control of the infection is at present troublesome because of the high number of re-infections which is related also to the behaviour of infected individuals who do not take the necessary care in avoiding the transmission of the disease for the whole duration of the infection. On the other hand, there is an increasing resistance of the causative organism to the commonly used antibiotics so that new associations of antibiotics and longer treatments become necessary. Therefore there is an increasing need for new antibiotic substances which might be effective in curing gonococcal infections, and in particular *Neisseria gonorrhoeae* infections, with a limited number of administrations, or even with a single dose administration.

In general for antibacterial treatment antibiotic A 40926 complex, one of its factors A, B, B_0, PA and PB as well as the non-toxic pharmaceutically acceptable salts thereof or mixture thereof, can be administered by different routes such as topically or parenterally. The parenteral administration is, in general, the preferred route of administration.

Compositions for injection may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain adjuvants such as suspending, stabilizing and/or dispersing agents.

Alt rnatively, the active ingredient may be in powder form for rec nstitution at the tim of delivery when a suitable vehicl , such as sterile water, is added th reto.

D pending on the route f administration, these comp unds can be formulated into various d sag forms.

In some instances, it may be possible to f rmulate the compounds of the invention in ent ric-coated

dosage forms for oral administration which may be prepared as known in the art (see for instance "Remington's Pharmaceutical Sciences", fifteenth edition, Mack Publishing Company, Easton, Pennsylvania, USA, page 1614).

This could be especially the case when the abs rption of the antimicrobial substance in the enteric tract is particularly desired while passing unaltered through the gastric tract.

The amount of active principle to be administered depends on various factors such as the size and condition of the subject to be treated, the route and frequency of administration, and the causative agent involved.

The antibiotic substances of the present invention, namely antibiotic A 40926 complex, its factors A, PA, B, B_0 and PB, and the physiologically acceptable salts thereof, are generally effective at a daily dosage of between about 0.5 and 50 mg of active ingredient per kilo of patient body weight, optionally divided into 1 to 4 administrations per day.

Particularly desirable compositions are those prepared in dosage units containing from about 100 to about 5,000 mg per unit.

However, when used in the single-dose treatment of gonorrhea, higher minimum doses of antibiotic A 40926 complex, its factors A, PA, B, B_0 or PB, generally ranging between 0.5 and 50 mg/kg, should be employed in order to maintain an effective blood level of the drug over an extended period of time.

Furthermore, in the treatment of gonorrhea, a sustained-action parenteral dosage form is preferably employed. Sustained-action formulations can be prepared based on different mechanisms and methods, as known in the art.

A preferred method for preparing a sustained-action formulation containing antibiotic A 40926 complex, its factors A, B, B_0, PA and PB involves the use of a water insoluble form of this antibiotic suspended in an aqueous or oily medium.

These forms, i.e. either as an insoluble salt or as the free acid, in fact, are released very slowly upon intramuscular injection, because of their low water-solubility, thus giving substained blood levels of the antibiotic substance.

Preparation of pharmaceutical compositions:

A unit dosage form for intramuscular injection is prepared with 5 ml of sterile suspension USP containing 8% propylene glycol and 1,000 mg of antibiotic A 40926 factor A.

A unit dosage form for intramuscular injection is prepared with 5 ml of sterile suspension USP containing 8% propylene glycol and 500 mg of antibiotic A 40926 factor B.

A unit dosage form for intramuscular injection is prepared with 2,000 mg of antibiotic A 40926 factor B sodium salt suspended in 5 ml of sterile water for injection.

Furthermore, the antibiotics of the invention are useful for suppressing the growth of *Clostridium difficile* which causes pseudomembranous colitis in the intestine. The antibiotic could be used in the treatment of pseudomembranous colitis by the oral administration of an effective dose of the antibiotic or a pharmaceutically-acceptable salt thereof, prepared in a pharmaceutically-acceptable dosage form. For such use, the antibiotic can be administered in gelatin capsules or in liquid suspension.

Besides their activity as medicaments, the compounds of the present invention can be used as animal growth promoters.

For this purpose, one or more of the compounds of the invention is administered orally in a suitable feed. The exact concentration employed is that which is required to provide for the active agent in a growth promotant effective amount when normal amounts of feed are consumed.

The addition of the active compounds of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compounds in an effective amount and incorporating the premix into the complete ration.

Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed.

The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W. H. Freedman and Co., S. Francisco, USA, 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Oregon, USA, 1977) and are incorporated herein by reference.

The following Examples further illustrate the invention and, as such, should not be construed as limiting its scope.

Example 1:

Fermentation of *Actinomadura sp.* ATCC 39727

A culture of antibiotic A 40926 producing strain (*Actinomadura sp.* ATCC 39727) is grown on oatmeal agar slant f r 2—3 weeks at 28°C and used to in culate a 500 ml Erlenmeyer flask c ntaining 100 ml of medium composed of 0.5% meat extract, 0.5% autolyzed yeast, 0.5% peptone, 0.3% casein hydrolysate, 2% glucose, 0.15% NaCl (pH 7.5 bef re sterilization).

The flask is incubated at 28°C on a rotary shaker at 200 rpm for about 72 h and then the culture is transferr d to a ferment r c ntaining 4 l of the above m dium. This cultur is grown at 28°C for ab ut 72 h

with air-flow of about two liters per minute and stirring at about 900 rpm. Then, it is used to inoculate a 200 l fermentor of the same medium. This fermentor is aerated with 100 l per minute of sterile air and is stirred at 250 rpm at about 28°C. The antibiotic production is monitored by the paper-disc agar diffusion method using *B. subtilis* on a minimal medium as the test organism. The maximum activity is obtained after 72—96 h.

Example 2:
Recovery of antibiotic A 40926

A) The fermentation broth is cooled to 4°C, brought to pH 9.5 and stirred. After about 1 h it is filtered and the filtrate is adjusted to pH about 3.5 with an aqueous mineral acid. The mixture is stirred for 30 min at 4°C and then filtered with (Hyflo-FloMa®) filter aid. The clear filtrate is discharged and the filter cake is suspended in deionized water, adjusted to pH about 8.5, stirred and then filtered. The recovered cake is subjected to the same procedure. The pooled filtrates contain antibiotic A 40926.

B) Swollen D-Ala-D-Ala-ε-aminocaproyl-Sepharose modified matrix (2 l) is added to the fermentation broth obtained according to Example 1 (after filtering it and bringing the pH of the clear filtrate to about 8.5) or to the pooled filtrate obtained according to the above Example 2A. After stirring overnight at room temperature, the resin is recovered by filtration and is washed sequentially with about 2×10 l of 0.45 mM HCl-TRIS buffer pH 7.5 (TRIS=2 - amino - 2 - hydroxymethyl - 1,3 - propanediol) which contains 5% (w/v) NaCl and then with distilled water (4×20 l).

The A 40926 antibiotic is eluted from the resin with 1% (w/v) ammonia hydrate (2×20 l). The eluates are left overnight at room temperature and then concentrated to a small volume (about 2.5 l). Water is eliminated by azeotropical distillation with n-butanol. Petroleum ether is then added, precipitating 3.4 g of crude antibiotic A 40926 complex.

Example 3:
Purification of antibiotic A 40926 complex AB

Crude antibiotic A 40926 complex obtained essentially following the procedure of the above Example 2, (750 mg; HPLC titre 70%) is dissolved in 400 ml of water, adjusted to pH 7.5 and filtered. The filtrate is then subjected to affinity chromatography on a D - Ala - D - Ala - ε - aminocaproyl - Sepharose column (50 ml of swollen resin; bed height=5 cm). The column, equilibrated with 0.16% (w/v) ammonia containing 2 M NaCl adjusted to pH 7.5 with HCl, is developed sequentially with the following three buffer solutions:

buffer A:
0.16% (w/v) ammonia containing 2M NaCl adjusted to pH 7.5 with HCl (2.6 column bed volumes);

buffer B:
0.16% (w/v) ammonia containing 2M NaCl adjusted to pH 9.5 with HCl (16 column bed volumes);

buffer C:
1% (w/v) aqueous ammonia pH 11.4 (2.6 column bed volumes).

Buffer C elutes antibiotic A 40926 complex AB in a single fraction. This eluted fraction is adjusted to pH 7.0 and reapplied to the same affinity column buffered with 10 mM TRIS-HCl pH 7.0. The column is washed with distilled water until desalting is complete.

The antibiotic is then eluted with 2 column bed volumes of 0.39% (w/v) aqueous ammonia pH 11.0.

The eluted fractions are concentrated to a small aqueous mixture and then freeze-dried. Pure antibiotic A 40926 complex AB (374 mg) is obtained.

Example 4:
Isolation of antibiotic A 40926 factors A and B

A) Antibiotic A 40926 complex as obtained according to Example 2 (3,3 g) or antibiotic A 40926 complex AB as obtained according to Example 3 (2.3 g) is suspended in 0.5 l of water, stirred and then filtered. The clear filtrate is applied to a silanized silica gel column (200 g; bed h 18 cm; silanized Silicagel 60; 70—230 mesh, Merck Inc.) pre-equilibrated with solution A (0.001 M aqueous sodium EDTA containing 0.25% (w/v) $NaH_2PO_4 \cdot H_2O$ and 2.5% (w/v) NaCl adjusted to pH 6.0 with NaOH). The column is eluted with a linear gradient from 0% to 40% (v/v) of acetonitrile in solution A with a total volume of about 7 l in about 48 h. Fractions of about 15.5 ml are collected and assayed by bioassay on *Bacillus subtilis* and analyzed by HPLC. Fractions having a similar antibiotic content are pooled. Fractions No. 310—330 and No. 348—365 contained the antibiotic substances denominated, respectively, A 40926 factor A and A 40926 factor B.

B) The pooled fractions containing the single A 40926 factors A and B are concentrated under reduced pressure to remove acetonitrile, diluted with water (about twice the volume of the initial solutions) and applied to a silanized silica gel c lumn f the type described above (volume of the swollen matrix: 50 ml; bed height of 15 cm). The column is washed with deionized water until desalting is complete and finally devel p d with acet nitrile/water 60:40 (v/v).

The eluted fracti ns are concentrated under reduced pressure and the r sidues are freeze-dried to btain 134 mg of antibi tic A 40926 factor A from the first gr up of eluted fracti ns (fractions 310—330

above) and 206 mg of A 40926 factor B from the second group of eluted fractions (fractions 348—365, above).

Example 5:
Isolation of antibiotic A 40926 factor PA and factor PB

By essentially following the procedure of Example 2A and the first steps of the procedure of Example 2B, the antibiotic linked to the resin is eluted with 1% (w/v) ammonia hydrate (2×20 l). The eluates are adjusted to pH 7.8 with sulfuric acid and concentrated to a small volume under vacuum by azeotropical distillation with n-butanol to obtain an aqueous concentrate which is then filtered on paper. The recovered filtrate contains antibiotic A 40926 factor PA, A 40926 factor PB and minor amounts of A 40926 factor A and factor B (HPLC). A sample (10 ml) of this aqueous concentrate containing about 50 mg/ml of pure antibiotic A 40926 complex (HPLC analysis) is filtered on 5 micrometer pore-size filter (Acrodisc®; Gelman Science Inc.) and then applied to a stainless steel column (diameter=2 cm) containing 20 g of an octadecyl silyl reverse-phase silica gel (Lichrisorb RP 18, Merck Inc.; particle size 10 μm). The silica gel is then packed under moderate pressure (nominal pressure about 14 bar) in a stainless steel column of a Chromatospac Modulprep apparatus (Yoben Yvon, France) and equilibrated with a mixture consisting of acetonitrile and 18 mM sodium phosphate buffer pH 6.0, 25:75 (v/v). The elution is carried out using the same solvent mixture used for the equilibration at a flow rate of about 10.5 ml/min. The eluate is monitored by bioassay on *Bacillus subtilis* and by HPLC.

Those fractions having similar antibiotic content are pooled and the homogeneous fractions of 5 chromatographic runs are concentrated to evaporate the organic solvent.

The resulting solution is diluted with aqueous 1M sodium chloride to twice the original volume and is applied to a silanized silica gel column (50 g; bed height 5 cm; Silanized silica gel 60; Merck Inc.) equilibrated with water.

The column is washed with deionized water until desalting is complete (no AgCl precipitation in the eluates after addition of aqueous $AgNO_3$) and then eluted with acetonitrile:water 1:1 (v/v). The eluates having similar antibiotic content (HPLC analysis) are pooled, concentrated to a small volume by azeotropical distillation with n-butanol to obtain an aqueous phase which is then freeze-dried. Yields:

antibiotic A 40926 factor PA: 55 mg
antibiotic A 40926 factor PB: 51 mg
antibiotic A 40926 factor A: 38 mg
antibiotic A 40926 factor B_0: 33 mg

Example 6:
Alternative method for isolating antibiotic A 40926 factor B

The pooled concentrate of two preparations made according to Example 2 (the last step) is filtered and the filtrate is applied to a silanized silica gel chromatography column; (400 g; bed h 30 cm; Silicagel 60, 70—230 mesh, Merck Inc.) pre-equilibrated with water.

The column is rinsed with water (6 l) and the adsorbed antibiotic is eluted with acetonitrile/water according to the following sequence:

2.7 l 5% (v/v) acetonitrile in water
1.6 l 10% (v/v) acetonitrile in water
2.97 l 15% (v/v) acetonitrile in water
3.15 l 20% (v/v) acetonitrile in water

Fractions of about 18 ml are collected. The activity of the eluted fractions is tested by paper-disc bioassay on susceptible microorganisms such as *B. subtilis* and analyzed by HPLC. Fractions with similar antibiotic content are pooled (fractions 472—526) and concentrated under reduced pressure. n-Butanol is added to this concentrate to azeotropically remove water. The butanolic solution which remains is in turn concentrated to a small volume to precipitate antibiotic A 40926 factor B (1.4 g). This product is washed with petroleum ether under stirring and collected by filtration (three times). Upon drying under vacuum 760 mg of A 40926 factor B are obtained.

By resubmitting antibiotic A 40926 factor B to the above column chromatography a product (540 mg) antibiotic A 40926 factor B_0 is obtained which has the same physico-chemical characteristics reported above for antibiotic A 40926 factor B except that it shows only a peak at HPLC analysis, namely the peak with retention time of 1.22 relative to Teicoplanin A_2 component 2.

Example 7:
Transformati n of antibi tic A 40926 factor PA and antibiotic A 40926 factor PB into antibi tic A 40926 fact r A and factor B, respectively

Antibi tic A 40926 fact r PA and antibiotic A 40926 factor PB (50 mg) are separately dissolved in 2.5 ml of aqueous 1% (w/v) NH_4OH and the resulting solutions ar kept f r about 24 h at room temperature with stirring. Antibiotic A 40926 factor A is obtained from the solution originally containing antibiotic A 40926

factor PA, and antibiotic A 40926 factor B is obtained fr m the solution originally containing antibiotic A 40926 fact r PB by r moving water by az otropic distillation with n-butanol, precipitating with ethyl ether and collecting the precipitate by filtration (yield about 75%).

Preparation of D-Ala-D-Ala-Sepharose

Activated CH-Sepharose 4B (Pharmacia Fine Chemicals) (1 g) is swollen for 15 minutes in 1 mM ice cold hydrochloric acid and washed with the same solution. The obtained gel (about 3 ml) is mixed with a solution of D-alanyl-D-alanine (30 mg) in 0.5 M sodium chloride and 0.1 M sodium bicarbonate buffer at pH 8.

The mixture is rotated end-over-end for 1 hour at room temperature.

After the coupling reaction is completed, the ligand excess is washed off with the buffer. The unlinked activated groups of the dextrane support are blocked by treating them with 1 M ethanolamine hydrochloride at pH 9 for 1 hour.

Then the Sephadex-ε-aminocaproyl-D-alanyl-D-alanine modified matrix is recovered by filtration and thoroughly washed alternatingly with 0.5 M sodium chloride and 0.1 M sodium acetate pH 4, and with 0.5 M sodium chloride and 0.1 M tris(hydroxymethyl)aminomethane buffer pH 8. (four times).

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. An antibiotic substance selected from antibiotic A 40926 complex, antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA, antibiotic A 40926 factor PB, a mixture thereof and the addition salts thereof which are characterized as following:

Antibiotic A 40926 factor A in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	281
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) methanol	282
e) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}):

3700—3100, 3100—2800 (nujol); 1655; 1620—1560; 1510; 1480—1410 (nujol); 1375 (nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (nujol).

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) at 270 MHz recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

δ0.86 (t's, 6H); 1.21 (~11H); 1.43 (2H); 2.01 (2H); 2.31—2.34 (3H); 4—6.2 (~16H); 6.2—8 (~23H); 8.44, 9.22, 9.66 (broad bands; mobile protons)

2.5—4: interference from H_2O peaks.

D) retention-time (R_t) of 0.60 relative to Testosterone when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradi nt from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Testosterone (Roussel Uclaf)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 4.6%) which indicates the following approximate percentage composition (average): carbon 55.82%; hydrogen 5.17%; nitrogen 6.31%; chlorine (total) 4.24%; chlorine (ionic) 0.37%. Inorganic residue at 900°C in the air: 1.2%.

F) acid-base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl which indicates four ionizable functions having the following pk_{MCS}: 4.6, 5.6, 7.2, 9.2.

G) R_f value of 0.24 and a R_f value relative to Teicoplanin A_2 component 2 of 0.70 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1716 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1717.

Antibiotic A 40926 factor B in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^1H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):
δ0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons)
2.5—4 interference from H_2O peaks.

D) Ret ntion times (R_t) of 1.22 and 1.27 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyz d by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 μm)

elu nt A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 9.6%) indicates the following approximate percentage composition (average):

carbon 54.09%; hydrogen 5.13%; nitrogen 6.34%; chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl (pH 2.7) which indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1731.

Antibiotic A 40926 factor B_0 in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) infrared absorption spectrum which exhibits the f ll wing abs rption maxima (cm^{-1}):
3700—3080, 3080—2700 (nuj l); 1720—1625; 1625—1560; 1505; 1460 (nuj l); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^{1}H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^{1}H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxid) using TMS as the internal standard (0.00 ppm), (δ=ppm):

δ 0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons)

2.5—4 interference from H_2O peaks.

D) Retention time (R_t) of 1.22 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC) under the following conditions:

column:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 9.6%) indicates the following approximate percentage composition (average):

carbon 54.09%; hydrogen 5.13%; nitrogen 6.34%; chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the M+H$^{\oplus}$ peak at 1731.

Antibiotic A 40926 factor PA in the non-salt form:

A) An ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	283 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3100; 3000—2800 (nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (nujol); 1375 (nujol); 1260, 1250—950; 845; 805; 720 (nujol)

C) ^{1}H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^{1}H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

0.86, d's (CH_3); 1.15—1.22, m $(CH_2)_n$; 1.41, m (CH_2); 2.01, s (CH_3); 2.01, m (CH_2); 2.28, s (N-CH_3); 4.26—5.96, br (peptidic and aromatic CH's); 6.33—7.73 br (aromatic CH's and peptidic NH's).

br = broad
d = doublet
dd = doublet of doublets
m = multiplet
s = singlet
t = triplet

D) retention time (R_t) of 1.15 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.62 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acet nitrile	30

using silanized silica gel 60 F_{254} Merck plates (lay r thickness 0.25 mm)

Visualization:

—U.V. light

—Yellow c lor with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))

—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

F) MW of about 1758 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1761. The operative conditions of the FAB-MS analysis were the following:

Instrument:

VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:

Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol-glycerol 1/1 (v/v).

Antibiotic A 40926 factor PB in the non-salt form:

A) An ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}):

3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (nujol); 1375 (nujol); 1320—1270; 1230—1190; 1150, 1120—920; 845; 810; 720 (nujol)

C.1) ^{1}H-NMR spectrum which exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):

0.84, d (isopropyl CH_3's); 1.17 m $(CH_2)_n$; 1.43, m (CH_2), 1.99, s (CH_3); 2.01, m (CH_2); 2.31, s (N-CH_3); 2.79, dd (C-H); 3.70, m (C-H); 4.06—6.02, br (peptidic and aromatic CH's); 6.45—7.74, br (aromatic CH's and peptidic NH's); 8.19—9.99, br (peptidic NH's and phenolic OH's)

C.2) ^{1}H-NMR spectrum which exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 plus CF_3COOD using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):

0.84, d (isopropyl CH_3's); 1.13, m $(CH_2)_n$; 1.40, m (CH_2); 1.98, s (CH_3); 2.00, m (CH_2); 2.92, dd (C-H); 3.29—3.71, m (sugar C-H's); 4.07—6.09, s and m (peptidic and aromatic CH's); 6.45—7.83, s and m (aromatic CH's and peptidic NH's); 8.17—10.38 (peptidic NH's, phenolic OH's).

D) retention times (R_t) of 1.27 and 1.32 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:

Ultrasphere® ODS (5 μm) Altex (Beckman 4.6 mm (i.d.)×250 mm

pre-column:

Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

eluti n:

lin ar gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:

1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.
F) MW of about 1772 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1776. The operative conditions of the FAB-MS analysis were the following:

Instrument:
VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:
Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol-glycerol 1/1 (v/v).

2. A process for preparing a compound of Claim 1 which comprises cultivating the strain *Actinomadura sp.* ATCC 39727, or an antibiotic A 40926-producing mutant or variant thereof, under submerged aerobic conditions in the presence of assimilable sources of carbon, nitrogen and inorganic salts, recovering and isolating said antibiotic from the fermentation broths and transforming it into the desired salt, if required.
3. A process as in Claim 2 wherein the strain is cultivated at a temperature between 20°C and 40°C.
4. A process as in Claim 2 wherein the temperature is between 24°C and 35°C.
5. A process as in Claim 2 wherein the recovery and isolation of the antibiotic substances is obtained by submitting the filtered fermentation both to an affinity chromatography on immobilized D-Alanyl-D-Alanine followed by partition, reverse-phase or ion-exchange chromatography.
6. A process according to Claim 2 wherein the recovery of the antibiotic substances includes:
a) submitting the fermentation broth to affinity chromatography on immobilized D-Alanyl-D-Alanine
b) rapidly neutralizing the pooled antibiotic containing eluted fractions and
c) optionally isolating the antibiotic A 40926 factors PA, PB, A, B and B_0 by means of reverse-phase liquid chromatography on silanized silica gel.
7. A process according to Claim 2 for preparing a compound selected from antibiotic A 40926 complex, antibiotic A 40926 factor A, factor B and factor B_0 which comprises:
a) making the fermentation mass basic at a pH between 8.5 and 10.5
b) filtering
c) acidifying the clear filtrate to pH 2.5—4.5
d) filtering and discharging the filtrate
e) suspending the filter cake in water and making it basic at a pH between 8 and 9
f) after recovering the crude antibiotic A 40926 complex by filtration, subjecting it to affinity chromatography on immobilized D-Alanyl-D-Alanine
g) optionally isolating antibiotic A 40926 factor A and factor B by means of partition, reverse-phase or ion-exchange chromatography, and
h) subjecting antibiotic A 40926 factor B to a further affinity chromatography procedure when antibiotic A 40926 factor B_0 is desired.
8. A process according to Claim 7 wherein the chromatography technique is reverse-phase chromatography and the stationary phase is selected from silanized silica gel and non-functionalized polystyrene resins.
9. A process according to Claim 7 wherein the stationary phase is silanized silica gel pre-equilibrated with a buffered solution at a pH between 4 and 9 and the eluent is a linear gradient mixture of a polar water-miscible solvent in the same buffered solution.
10. A comp und f Claim 1 f r use as a medicine.
11. A pharmaceutical composition which comprises a compound of Claim 1 in admixture with a pharmaceutically-acceptable vehicle.
12. The strain *Actinomadura sp.* ATCC 39727.
13. A biol gically pure culture f the strain *Actinomadura sp.* ATCC 39727 or an antibiotic A 40926

producing mutant or variant thereof, capable of producing a compound of claim 1 when cultivated under submerged aerobic conditions in the presence of assimilable sources of carbon, nitrogen and inorganic salts.

Claims for the Contracting State: AT

1. A process for preparing an antibiotic substance selected from antibiotic A 40926 complex, antibiotic A 40926 factor A, antibiotic A 40926 factor B, antibiotic A 40926 factor B_0, antibiotic A 40926 factor PA, antibiotic A 40926 factor PB, a mixture thereof and the addition salts thereof which are characterized as following:

Antibiotic A 40926 factor A in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	281
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) methanol	282
e) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3100, 3100—2800 (nujol); 1655; 1620—1560; 1510; 1480—1410 (nujol); 1375 (nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (nujol).

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) at 270 MHz recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm): δ 0.86 (t's, 6H); 1.21 (~11H); 1.43 (2H); 2.01 (2H); 2.31—2.34 (3H); 4—6.2 (~16H); 6.2—8 (~23H); 8.44, 9.22, 9.66 (broad bands; mobile protons)
2.5—4: interference from H_2O peaks.

D) retention-time (R_t) of 0.60 relative to Testosterone when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Testosterone (Roussel Uclaf)

E) elemental analysis, after the sample has been previ usly dried at about 140°C under inert atmosphere (Δw 4.6%) which indicates the following approximate percentage composition (average): carbon 55.82%; hydrogen 5.17%; nitrogen 6.31%; chlorine (total) 4.24%; chlorine (ionic) 0.37%. Inorganic residue at 900°C in the air: 1.2%.

F) acid-base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl which indicates four ionizable functions having the following pk_{MCS}: 4.6, 5.6, 7.2, 9.2.

G) R_f value of 0.24 and a R_f value relative to Teicoplanin A_2 component 2 of 0.70 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:

—U.V. light

—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))

—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1716 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1717.

Antibiotic A 40926 factor B in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) Infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^1H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

δ 0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons)

2.5—4 interference from H_2O peaks.

D) Retention times (R_t) of 1.22 and 1.27 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:

Ultrasphere® ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:

Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

elu nt B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphere (Δw 9.6%) indicates the following approximate percentage composition (average): carbon 54.09%; hydrogen 5.13%; nitrogen 6.34% chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl (pH 2.7) which indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1731.

Antibiotic A 40926 factor B_0 in the non-salt form:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) phosphate buffer pH 7.38	281 300 (shoulder)
c) 0.1 N sodium or potassium hydroxide	300
d) phosphate buffer pH 9.0	283 300 (shoulder)
e) methanol	282

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^1H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm):

δ 0.85 (d, isopropyl CH_3's); 1.15 (~13H); 1.44 (~2H); 2.02 (2H); 2.32—2.35 (3H); 4—6.1 (~16H); 6.1—8 (~23H); 8.52, 9.30, 9.68 (broad bands; mobile protons)

2.5—4 interference from H_2O peaks.

D) Ret ntion time (R_t) of 1.22 relative to Teicoplanin A_2 compon nt 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee Labs RP 18 (5 µm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) elemental analysis, after the sample has been previously dried at about 140°C under inert atmosphre (Δw 9.6%) indicates the following approximate percentage composition (average): carbon 54.09%; hydrogen 5.13%; nitrogen 6.34%; chlorine (total) 4.12%; chlorine (ionic) 0.39%. Inorganic residue at 900°C in the air: 5%.

F) acid base titration profile in 2-methoxyethanol (MCS):water, 4:1 upon titration with KOH after addition of an excess of HCl indicates four ionizable functions having the following pk_{MCS}: 4.5, 5.6, 7.2, 9.2.

G) R_f value of 0.21 and a R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

H) MW of about 1730 desumed from a FAB-MS spectrum showing the $M+H^{\oplus}$ peak at 1731.

Antibiotic A 40926 factor PA in the non-salt form:

A) An ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	283 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (nujol); 1375 (nujol); 1260, 1250—950; 845; 805; 720 (nujol)

C) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) in the 270 MHz ^1H-NMR recorded in DMSO d_6 (hexadeuterodimethylsulfoxid) using TMS as the internal standard (0.00 ppm), (δ=ppm):

0.86, d's(CH_3); 1.15—1.22, m $(CH_2)_n$; 1.41, m (CH_2); 2.01, s (CH_3); 2.01, m (CH_2); 2.28, s (N-CH_3); 4.26—5.96, br (peptidic and aromatic CH's); 6.33—7.73 br (aromatic CH's and peptidic NH's).

br = broad
d = doublet
dd = doublet of doublets
m = multiplet
s = singlet
t = triplet

D) retention time (R_t) of 1.15 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.62 in the following chromatographic system:

5% (w/v) aqueous Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:
—U.V. light
—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

F) MW of about 1758 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1761. The operative conditions of the FAB-MS analysis were the following:

instrument:
VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:
Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol-glycerol 1/1 (v/v).

Antibiotic A 40926 factor PB in the non-salt form:

A) An ultraviolet absorption spectrum which exhibits the following absorption maxima:

	λ max (nm)
a) 0.1 N HCl	282
b) 0.1 N potassium hydroxide	300
c) phosphate buffer pH 7.38	282 300 (shoulder)
d) phosphate buffer pH 9.0	282 300 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima (cm^{-1}): 3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (nujol); 1375 (nujol); 1320—1270; 1230—1190; 1150, 1120—920; 845; 810; 720 (nujol)

C.1) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):

0.84, d (isopropyl CH_3's); 1.17, m $(CH_2)_n$; 1.43, m (CH_2); 1.99, s (CH_3); 2.01, m (CH_2); 2.31, s (N-CH_3); 2.79, dd (C-H); 3.70, m (C-H); 4.06—6.02, br (peptidic and aromatic CH's); 6.45—7.74, br (aromatic CH's and peptidic NH's); 8.19—9.99, br (peptidic NH's and phenolic OH's)

C.2) ^1H-NMR spectrum which exhibits the following groups of signals (in ppm) at the 270 MHz in DMSO d_6 plus CF_3COOD using TMS as the internal standard (0.00 ppm), (δ=ppm) multiplicity; (attribution):

0.84, d (isopropyl CH's); 1.13, m $(CH_2)_n$; 1.40, m (CH_2); 1.98, s (CH_3); 2.00, m (CH_2); 2.92, dd (C-H); 3.29—3.71, m (sugar C-H's); 4.07—6.09, s and m (peptidic and aromatic CH's); 6.45—7.83, s and m (aromatic CH's and peptidic NH's); 8.17—10.38 (peptidic NH's, phenolic OH's).

D) retention times (R_t) of 1.27 and 1.32 relative to Teicoplanin A_2 component 2 (R_t=20.3 min) when analyzed by reverse phase HPLC under the following conditions:

column:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4.6 mm (i.d.)×250 mm

pre-column:
Brownlee® Labs RP 18 (5 μm)

eluent A:

CH_3CN	10%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	pH 6.0

eluent B:

CH_3CN	70%	adjusted at
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	pH 6.0

elution:
linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate:
1.8 ml/min

U.V. detector:
254 nm

internal standard:
Teicoplanin A_2 component 2 (Gruppo Lepetit S.p.A.)

E) R_f value relative to Teicoplanin A_2 component 2 of 0.53 in the following chromatographic system:

5% (w/v) aque us Na_2SO_4	70
acetonitrile	30

using silanized silica gel 60 F_{254} Merck plates (layer thickness 0.25 mm)

Visualization:

—U.V. light

—Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))

—Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

F) MW of about 1772 desumed from a FAB-MS spectrum showing a cluster of peaks having the most intense peak at 1776. The operative conditions of the FAB-MS analysis were the following:

Instrument:

VG Mod ZAB SE equipped with FAB gun Ion Tech

Conditions:

Positive FAB, Xe Accelerating voltage, 8KV Matrix: Thioglycerol-glycerol 1/1 (v/v)

which comprises cultivating the strain *Actinomadura sp.* ATCC 39727, or an antibiotic A 40926-producing mutant or variant thereof, under submerged aerobic conditions in the presence of assimilable sources of carbon, nitrogen and inorganic salts, recovering and isolating said antibiotic from the fermentation broths and transforming it into the desired salt, if required.

2. A process as in Claim 1 wherein the strain is cultivated at a temperature between 20°C and 40°C.

3. A process as in Claim 1 wherein the temperature is between 24°C and 35°C.

4. A process as in Claim 1 wherein the recovery and isolation of the antibiotic substances is obtained by submitting the filtered fermentation broth to an affinity chromatography on immobilized D-Alanyl-D-Alanine followed by partition, reverse-phase or ion-exchange chromatography.

5. A process according to Claim 1 wherein the recovery of the antibiotic substances includes:

a) submitting the fermentation broth to affinity chromatography on immobilized D-Alanyl-D-Alanine

b) rapidly neutralizing the pooled antibiotic containing eluted fractions and

c) optionally isolating the antibiotic A 40926 factors PA, PB, A, B and B_0 by means of reverse-phase liquid chromatography on silanized silica gel.

6. A process according to Claim 1 for preparing a compound selected from antibiotic A 40926 complex, antibiotic A 40926 factor A, factor B and factor B_0 which comprises:

a) making the fermentation mass basic at a pH between 8.5 and 10.5

b) filtering

c) acidifying the clear filtrate to pH 2.5—4.5

d) filtering and discharging the filtrate

e) suspending the filter cake in water and making it basic at a pH between 8 and 9

f) after recovering the crude antibiotic A 40926 complex by filtration, subjecting it to affinity chromatography on immobilized D-Alanyl-D-Alanine

g) optionally isolating antibiotic A 40926 factor A and factor B by means of partition, reverse-phase or ion-exchange chromatography, and

h) subjecting antibiotic A 40926 factor B to a further affinity chromatography procedure when antibiotic A 40926 factor B_0 is desired.

7. A process according to Claim 6 wherein the chromatographic technique is reverse-phase chromatography and the stationary phase is selected from silanized silica gel and non-functionalized polystyrene resins.

8. A process according to Claim 6 wherein the stationary phase is silanized silica gel pre-equilibrated with a buffered solution at a pH between 4 and 9 and the eluent is a linear gradient mixture of a polar water-miscible solvent in the same buffered solution.

9. Use of a compound of Claim 1 for preparing a medicament for antibacterial use.

Patentansprüche für die Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Eine antibiotische Substanz, ausgewählt aus Antibiotikum-A 40926-Komplex, Antibiotikum-A 40926-Faktor A, Antibiotikum-A 40926-Faktor B, Antibiotikum-A 40926-Faktor B_0, Antibiotikum-A 40926-Faktor PA, Antibiotikum-A 40926-Faktor PB, einem Gemisch davon, und den Säureadditionssalzen davon, welche antibiotischen Substanzen wie folgt gekennzeichnet sind:

Antibiotikum-A 40926-Faktor A in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

		λ max (nm)
a)	0,1N HCl	281
b)	Phosphatpuffer pH 7,38	281 300 (Schulter)
c)	0,1N Natrium- oder Kaliumhydroxid	300
d)	Methanol	282
e)	Phosphatpuffer pH 9,0	282 300 (Schulter)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt: 3700—3100, 3100—2800 (Nujol); 1655; 1620—1560; 1510; 1480—1410 (Nujol); 1375 (Nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (Nujol).

C) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:
δ 0,86 (t's, 6H); 1.21 (~11H); 1,43 (2H); 2,01 (2H); 2,31—2,34 (3H); 4—6,2 (~16H); 6,2—8 (~23H); 8,44, 9,22, 9,66 (breite Banden; mobile Protonen) 2,5—4: Interferenz aus H_2O-Peaks.

D) Retentionszeit (R_t) von 0,60 in bezug auf Testosteron bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere ODS (5 μm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee Labs RP 18 (5 μm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Testosteron (Roussel Uclaf)

E) Elementaranalyse, nachdem die Probe vorher bei etwa 140°C unter einer Inertatmosphäre (Δw 4,6%) getrocknet worden ist, wobei die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:
Kohlenstoff 55,82%; Wasserstoff 5,17%; Stickstoff 6,31%; Chlor (insgesamt) 4,24%; Chlor (ionisch) 0,37%.
Anorganischer Rückstand bei 900°C in Luft: 1,2%.

F) Säure-Base-Titrationsprofil in 2-Methoxyethanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses von HCl, welches Profil vier ionisierbare Funktionen mit den folgenden pk_{MCS}-Werten anzeigt: 4,6, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,24 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,70 in dem folgenden chromatographischen System:

5%ig s (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiert m Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:

—UV-Licht

—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))

—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1716, errechnet aus einem FAB-MS-Spektrum, das den M+H®-Peak bei 1717 zeigt.

Antibiotikum-A 40926-Faktor B in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) Phosphatpuffer pH 7,38	281 300 (Schulter)
c) 0,1N Natrium- oder Kaliumhydroxid	300
d) Phosphatpuffer pH 9,0	283 300 (Schulter)
e) Methanol	282

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt: 3700—3080, 3080—2700 (Nujol); 1720—1625; 1625—1560; 1505; 1460 (Nujol); 1375 (Nujol); 1295; 1230, 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (Nujol).

C) ^{1}H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^{1}H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:

δ 0,85 (d, Isopropyl CH_3's); 1,15 (~13H); 1,44 (~2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (~16H); 6,1—8 (~23H); 8,52, 9,30, 9,68 (breite Banden; mobile Protonen) 2,5—4 Interferenz von H_2O-Peaks.

D) Retentionszeiten (R_t) von 1,22 und 1,27 in bezug auf Teicoplanin A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkrehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teic planin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) Elementaranalyse, nachdem die Probe vorher bei etwa 140°C unter einer Inertatmosphäre (Δw 9,6%) getrocknet worden ist, wob i die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:

Kohlenstoff 54,09%; Wasserstoff 5,13%; Stickstoff 6,34%; Chlor (insgesamt) 4,12%; Chlor (ionisch) 0,39%. Anorganischer Rückstand bei 900°C in Luft: 5%.

F) Säure-Base-Titrationsprofil in 2-Methoxyethanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses von HCl (pH 2,7), welches Profil vier ionisierbare Funktionen mit den folgenden pk_{MCS}-Werten anzeigt: 4,5, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,21 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:

—UV-Licht

—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog, 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))

—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1730, errechnet aus einem FAB-MS-Spektrum, das den $M+H^{\oplus}$-Peak bei 1731 zeigt.

Antibiotikum-A 40926-Faktor B_0 in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) Phosphatpuffer pH 7,38	281 300 (Schulter)
c) 0,1N Natrium- oder Kaliumhydroxid	300
d) Phosphatpuffer pH 9,0	283 300 (Schulter)
e) Methanol	282

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:

3700—3080, 3080—2700 (Nujol); 1720—1625; 1625—1560; 1505; 1460 (Nujol); 1375 (Nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (Nujol).

C) ^{1}H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^{1}H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:

δ 0,85 (d, Isopropyl CH_3's); 1,15 (~13H); 1,44 (~2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (~16H); 6,1—8 (~23H); 8,52, 9,30, 9,68 (breite Banden; mobile Protonen)

2,5—4 Interferenz von H_2O-Peaks.

D) Retentionszeit (R_t) von 1,22 in bezug auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:

Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:

Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	ing st llt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Elulerung:

linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Det ktor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) Elementaranalyse, nachdem die Probe vorher bei etwa 140°C unter einer Intertatmosphäre (Δw 9,6%) getrocknet worden ist, wobei die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:

Kohlenstoff 54,09%; Wasserstoff 5,13%; Stickstoff 6,34%; Chlor (insgesamt) 4,12%; Chlor (ionisch) 0,39%. Anorganischer Rückstand bei 900°C in Luft: 5%.

F) Säure-Base-Titrationsprofil in 2-Methoxyethanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses von HCl, welches Profil vier ionisierbare Funktionen mit den folgenden pk_{MCS}-Werten anzeigt: 4,5, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,21 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographischem System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1730, errechnet aus einem FAB-MS-Spektrum, das den $M+H^{\oplus}$-Peak bei 1731 zeigt.

Antibiotikum-A 40926-Faktor PA in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) 0,1N Kaliumhydroxid	300
c) Phosphatpuffer pH 7,38	282 300 (Schulter)
d) Phosphatpuffer pH 9,0	283 300 (Schulter)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:

3700—3100, 3000—2800 (Nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (Nujol); 1375 (Nujol); 1260, 1250—950; 845; 805; 720 (Nujol)

C) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^1H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:

0,86, d's (CH_3); 1,15—1,22, m ($(CH_2)_n$); 1,41, m (CH_2); 2,01, s (CH_3); 2,01, m (CH_2); 2,28, s ($N-CH_3$); 4,26—5,96, br (peptidische und aromatische CH's); 6,33—7,73 br (aromatische CH's und peptidische NH's).

br = breit
d = Doublett
dd = Doublett von Doubletts
m = Multiplett
s = Singlett
t = Triplett

D) Retentionszeit (R_t) von 1,15 in bezug auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,62 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

F) Molekulargewicht von etwa 1758, errechnet aus einem FAB-MS-Spektrum, das einen Haufen von Peaks mit dem intensivsten Peak bei 1761 zeigt. Die Arbeitsbedingungen der FAB-MS-Analyse waren die folgenden:

Instrument:
VG Mod ZAB SE, ausgestattet mit FAB gun Ion Tech

Bedingungen:
Positive FAB, Xe Beschleunigungsspannung: 8kV; Matrix: Thioglycerin-Glycerin 1/1 (Vol./Vol.).

Antibiotikum-A 40926-Faktor PB in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) 0,1N Kaliumhydroxid	300
c) Phosphatpuff r pH 7,38	282 300 (Schult r)
d) Ph sphatpuffer pH 9,0	282 300 (Schult r)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:

3700—3100, 3000—2800 (Nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (Nujol); 1375 (Nujol); 1320—1270; 1230—1190; 1150; 1120—920; 845; 810, 720 (Nujol)

C.1) ^{1}H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz in DMSO d_6 (Hexadeuterodim thylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt; Multiplizität; (Zuschreibung):

0,84, d (Isopropyl CH_3's); 1,17, m $(CH_2)_n$; 1,43, m (CH_2); 1,99, s (CH_3); 2,01, m (CH_2); 2,31, s (N-CH_3); 2,79, dd (C-H); 3,70, m (C-H); 4,06—6,02, br (peptidische und aromatische CH's); 6,45—7,74, br (aromatische CH's und peptidische NH's); 8,19—9,99 br (peptidische NH's und phenolische OH's).

C.2) ^{1}H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz in DMSO d_6 plus CF_3 COOD unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt: Multiplizität; (Zuschreibung):

0,84, d (Isopropyl CH_3's); 1,13, m $(CH_2)_n$; 1,40, m (CH_2); 1,98, s (CH_3); 2,00, m (CH_2); 2,92, dd (C-H); 3,29—3,71, m (Zucker C-H's); 4,07—6,09, s und m (peptidische und aromatische CH's); 6,45—7,83, s und m (aromatische CH's und peptidische NH's); 8,17—10,38 (peptidische NH's, phenolische OH's).

D) Retentionszeiten (R_t) von 1,27 und 1,32, bezogen auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkrehrphase-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtillis ATCC 6633 auf Davis-Minimalmedium.

F) Molekulargewicht von etwa 1772, errechnet aus einem FAB-MS-Spektrum, das einen Haufen von Peaks mit dem intensivsten Peak bei 1776 zeigt. Die Arbeitsbedingungen der FAB-MS-Analyse waren die folgenden:

Instrument:
GB Mod ZAB SE, ausgestattet mit FAB gun Ion Tech

Bedingungen:
Positive FAB, Xe Beschleunigungsspannung, 8kV Matrix: Thioglycerin-Glycerin 1/1 (Vol./Vol.).

2. Ein Verfahren zur Herstellung einer Verbindung von Anspruch 1, welches das Kultivieren des Stammes Actinomadura sp. ATCC 39727, oder einer Antibiotikum-A 40926-produzierenden Mutante oder Variante davon, unter submersen, aeroben Bedingungen in Gegenwart von assimilierbaren Quellen von Kohlenstoff, Stickstoff und anorganischen Salzen, das Gewinnen und das Isolieren des genannten Antibiotikums aus den Fermentationsbrühen und erforderlichenfalls das Umwandeln desselben in das gewünschte Salz, umfaßt.

3. Ein Verfahren wie in Anspruch 2, wobei der Stamm bei einer Temperatur zwischen 20°C und 40°C kultiviert wird.

4. Ein Verfahren wie in Anspruch 2, wobei die Temperatur zwischen 24°C und 35°C liegt.

5. Ein Verfahren wie in Anspruch 2, wobei die Gewinnung und Isolierung der antibiotischen Substanzen dadurch erzielt wird, daß die filtrierte Fermentationsbrühe einer Affinitätschromatographie an immobilisiertem D-Alanyl-Alanin, gefolgt von Verteilungs-, Umkehrphasenoder Ionenaustauscherchromatographie, unterworfen wird.

6. Ein Verfahren nach Anspruch 2, wobei die Gewinnung der antibiotischen Substanzen umfaßt:

a) das Unterwerfen der Fermentationsbrühe der Affinitätschromatographie an immobilisiertem D-Alanyl-D-Alanin

b) das rasche Neutralisieren der vereinigten, Antibiotikumhältigen, eluierten Fraktionen, und

c) gegebenenfalls das Isolieren der Antibiotikum-A 40926-Faktoren PA, PB, A, B und B_0 mittels Umkehrphasen-Flüssigkeitschromatographie an silanisiertem Kieselsäuregel.

7. Ein Verfahren nach Anspruch 2 zur Herstellung einer Verbindung, ausgewählt aus Antibiotikum-A 40926-Komplex, Antibiotikum-A 40926-Faktor A, Faktor B und Faktor B_0, welches umfaßt:

a) das Basischstellen der Fermentationsmasse auf einen pH-Wert zwischen 8,5 und 10,5

b) das Filtrieren

c) das Ansäuern des klaren Filtrates auf einen pH-Wert von 2,5—4,5

d) das Filtrieren und das Verwerfen des Filtrates

e) das Suspendieren des Filterkuchens in Wasser und das Basischstellen dessselben auf einen pH-Wert zwischen 8 und 9

f) das Unterwerfen des rohen Antibiotikum-A 40926-Komplexes, nach dessen Gewinnung durch Filtration, der Affinitätschromatographie an immobilisiertem D-Alanyl-D-Alanin

g) gegebenenfalls das Isolieren von Antibiotikum-A 40926-Faktor A und Faktor B mittels Verteilungs-, Umkehrphasen- oder Ionenaustauscherchromatographie, und

h) das Unterwerfen von Antibiotikum-A 40926-Faktor B einer weiteren Affinitätschromatographieverfahrensweise, falls Antibiotikum-A 40926-Faktor B_0 erwünscht ist.

8. Ein Verfahren nach Anspruch 7, wobei die chromatographische Methode Umkehrphasenchromatographie ist, und die stationäre Phase aus silanisiertem Kieselsäuregel und nicht-funktionalisierten Polystyrolharzen ausgewählt ist.

9. Ein Verfahren nach Anspruch 7, wobei die stationäre Phase silanisiertes Kieselsäuregel ist, das mit einer gepufferten Lösung bei einem pH-Wert zwishen 4 und 9 vor-äquilibriert worden ist, und wobei das Eluierungsmittel ein Lineargradientengemisch eines polaren, wassermischbaren Lösungsmittels in der gleichen, gepufferten Lösung ist.

10. Eine Verbindung von Anspruch 1 zur Verwendung als ein Arzneimittel.

11. Eine pharmazeutische Zusammensetzung, welche eine Verbindung von Anspruch 1 im Gemisch mit einem pharmazeutisch annehmbaren Träger enthält.

12. Der Stamm Actinomadura sp. ATCC 39727.

13. Eine biologisch reine Kultur des Stammes Actinomadura sp. ATCC 39727, oder einer Antibiotikum-A 40926-produzierenden Mutante oder Variante davon, die, wenn sie unter submersen, aeroben Bedingungen in Gegenwart von assimilierbaren Quellen von Kohlenstoff, Stickstoff und anorganischen Salzen kultiviert wird, zur Produktion einer Verbindung von Anspruch 1 geeignet ist.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung einer antibiotischen Substanz, ausgewählt aus Antibiotikum-A 40926-Kompex, Antibiotikum-A 40926-Faktor A, Antibiotikum-A 40926-Faktor B, Antibiotikum-A 40926-Faktor B_0, Antibiotikum-A 40926-Faktor PA, Antibiotikum-A 40926-Faktor PB, einem Gemisch davon, und den Säureadditionssalzen davon, welche antibiotischen Substanzen wie folgt gekennzeichnet sind:

Antibiotikum-A 40926-Faktor A in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	281
b) Phosphatpuffer pH 7,38	281 300 (Schulter)
c) 0,1N Natrium- oder Kaliumhydroxid	300
d) Methanol	282
e) Phosphatpuffer pH 9,0	282 300 (Schulter)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:
3700—3100, 3100—2800 (Nujol); 1655; 1620—1560; 1510; 1480—1410 (Nujol); 1375 (Nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (Nujol).

C) ^{1}H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:
δ 0,86 (t's, 6H); 1,21 (~11H); 1,43 (2H); 2,01 (2H); 2,31—2,34 (3H); 4—6,2, (~16H); 6,2—8 (~23H); 8,44, 9,22, 9,66 (breite Banden; mobile Protonen) 2,5—4: Interferenz aus H_2O-Peaks.

D) Retentionszeit (R_t) von 0,60 in bezug auf Testosteron bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Testosteron (Roussel Uclaf)

E) Elementaranlyase, nachdem die Probe vorher bei etwa 140°C unter einer Interatmosphäre (Δw 4,6%) getrocknet worden ist, wobei die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:
Kohlenstoff 55,82%; Wasserstoff 5,17%; Stickstoff 6,31%; Chlor (insgesamt) 4,24%; Chlor (ionisch) 0,37%. Anorganischer Rückstand bei 900°C in Luft: 1,2 %.

F) Säure-Base-Titrati nsprofil in 2-M thoxy thanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses v n HCl, welches Profil vier ionisierbare Funktionen mit den f lgenden pk_{MCS}-Werten anzeigt: 4,6, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,24 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,70 in dem folgenden chromatographischen Syst m:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Ac tonitril	30

unter Verwendung von F_{254}-M rck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1716, errechnet aus einem FAB-MS-Spektrum, das den $M+H^{\oplus}$-Peak bei 1717 zeigt.

Antibiotikum-A 40926-Faktor B in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) Phosphatpuffer pH 7,38	281 300 (Schulter)
c) 0,1N Natrium- oder Kaliumhydroxid	300
d) Phosphatpuffer pH 9,0	283 300 (Schulter)
e) Methanol	282.

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:
3700—3080, 3080—2700 (Nujol); 1720—1625; 1625—1560; 1505; 1460 (Nujol); 1375 (Nujol); 1295; 1230, 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (Nujol).

C) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^1H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS also dem internen Standard (0,00 ppm), (δ=ppm) zeigt:
δ 0,85 (d, Isopropyl CH_3's); 1,15 (~13H); 1,44 (~2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (~16H); 6,1—8 (~23H); 8,52, 9,30, 9,68 (breite Banden; mobile Protonen) 2,5—4 Interferenz von H_2O-Peaks.

D) Retentionszeiten (R_t) von 1,22 und 1,27 in bezug auf Teicoplanin A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-K mp n nte 2 (Gruppe Lepetit S.p.A.)

E) Elementaranalyse, nachdem die Probe vorher bei etwa 140°C unter einer Inertatmosphäre (Δw 9,6%) getrocknet worden ist, wobei die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:

Kohlenstoff 54,09%; Wasserstoff 5,13%; Stickstoff 6,34%; Chlor (insgesamt) 4,12%; Chlor (ionisch) 0,39%. Anorganischer Rückstand bei 900°C in Luft: 5%.

F) Säure-Base-Titrationsprofil in 2-Methoxyethanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses von HCl (pH 2,7), welches Profil vier ionisierbare Funktionen mit den folgenden pk_{MCS}-Werten anzeigt: 4,5, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,21 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographichen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:

—UV-Licht

—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))

—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1730, errechnet aus einem FAB-MS-Spektrum, das den $M+H^{\oplus}$-Peak bei 1731 zeigt.

Antibiotikum-A 40926-Faktor B_0 in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) Phosphatpuffer pH 7,38	281 300 (Schulter)
c) 0,1N Natrium- oder Kaliumhydroxid	300
d) Phosphatpuffer pH 9,0	283 300 (Schulter)
e) Methanol	282

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:

3700—3080, 3080—2700 (Nujol); 1720—1625; 1625—1560; 1505; 1460 (Nujol); 1375 (Nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (Nujol).

C) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^1H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:

δ 0,85 (d, isopropyl CH_3's); 1,15 (~13H); 1,44 (~2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (~16H); 6,1—8 (~23H); 8,52, 9,30, 9,68 (breite Banden; mobile Protonen)

2,5—4 Interferenz von H_2O-Peaks.

D) Retentionszeit (R_t) von 1,22 in bezug auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:

Ultrasphere® ODS (5 μm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:

Brownlee® Labs RP 18 (5 μm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingest llt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% d s Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) Elementaranalyse, nachdem die Probe vorher bei etwa 140°C unter einer Interatmosphäre (Δw 9,6%) getrocknet worden ist, wobei die folgende annähernde prozentuelle Zusammensetzung (Durchschnitt) ermittelt wird:

Kohlenstoff 54,09%; Wasserstoff 5,13%; Stickstoff 6,34%; Chlor (insgesamt) 4,12%; Chlor (ionisch) 0,39%.

Anorganischer Rückstand bei 900°C in Luft: 5%.

F) Säure-Base-Titrationsprofil in 2-Methoxyethanol (MCS):Wasser, 4:1, bei der Titration mit KOH nach Zusatz eines Überschusses von HCl, welches Profil vier ionisierbare Funktionen mit den folgenden pk_{MCS}-Werten anzeigt: 4,5, 5,6, 7,2, 9,2.

G) R_f-Wert von 0,21 und ein R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog, 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

H) Molekulargewicht von etwa 1730, errechnet aus einem FAB-MS-Spektrum, das den $M+H^{\oplus}$-Peak bei 1731 zeigt.

Antibiotikum-A 40926-Faktor PA in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) 0,1N Kaliumhydroxid	300
c) Phosphatpuffer pH 7,38	282 300 (Schulter)
d) Phosphatpuffer pH 9,0	283 300 (Schulter)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:
3700—3100, 3000—2800 (Nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (Nujol); 1375 (Nujol); 1260, 1250—950; 845; 805; 720 (Nujol)

C) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei der 270 MHz ^1H-NMR, aufgezeichnet in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt:

0,86, d's (CH_3); 1,15—1,22, m $(CH_2)_n$; 1,41, m (CH_2); 2,01, s (CH_3); 2,01, m (CH_2); 2,28, s (N-CH_3); 4,26—5,96, br (peptidische und aromatische CH's); 6,33—7,73 br (aromatische CH's und peptidische NH's).

br = breit
d = Doublett
dd = Doublett v n Doubletts
m = Multiplett
s = Singlett
t = Triplett

D) Retentionszeit (R_t) von 1,15 in bezug auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säle:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2.5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,62 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

F) Molekulargewicht von etwa 1758, errechnet aus einem FAB-MS-Spektrum, das einen Haufen von Peaks mit dem intensivsten Peak bei 1761 zeigt. Die Arbeitungsbedingungen der FAB-MS-Analyse waren die folgenden:

Instrument:
VG Mod ZAB SE, ausgestattet mit FAB gun Ion tech

Bedingungen:
Positive FAB, Xe Beschleunigungsspannung: 8kV; Matrix: Thioglycerin-Glycerin 1/1 (Vol./Vol.).

Antibiotikum-A 40926-Faktor PB in der Nicht-Salz-Form:

A) Ultraviolettabsorptionsspektrum, welches die folgenden Absorptionsmaxima zeigt:

	λ max (nm)
a) 0,1N HCl	282
b) 0,1N Kaliumhydroxid	300
c) Ph sphatpuffer pH 7,38	282 300 (Schulter)
d) Phosphatpuffer pH 9,0	282 300 (Schulter)

B) Infrarotabsorptionsspektrum, welches die folgenden Absorptionsmaxima (cm^{-1}) zeigt:

3700—3100, 3000—2800 (Nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (Nujol); 1375 (Nujol); 1320—1270; 1230—1190; 1150; 1120—920; 845; 810, 720 (Nujol)

C.1) ^1H-NMR-Sp ktrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz in DMSO d_6 (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als dem internen Standard (0,00 ppm), (δ=ppm) zeigt; Multiplizität; (Zuschreibung):

0,84, d (Isopropyl CH_3's); 1,17, m $(CH_2)_n$; 1,43, m (CH_2), 1,99, s (CH_3); 2,01, m (CH_2); 2,31, s $(N\text{-}CH_3)$; 2,79, dd (C-H); 3,70, m (C-H); 4,06—6,02, br (peptidische und aromatische CH's); 6,45—7,74, br (aromatische CH's und peptidische NH's); 8,19—9,99, br (peptidische NH's und phenolische OH's)

C.2) ^1H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 270 MHz in DMSO d_6 plus CF_3COOD unter Verwendung von TMS als dem internen Standard (0.00 ppm), (δ=ppm) zeigt: Multiplizität; (Zuschreibung):

0,84, d (Isopropyl CH_3's); 1,13, m $(CH_2)_n$; 1,40, m (CH_2); 1,98, s (CH_3); 2,00, m (CH_2); 2,92, dd (C-H); 3,29—3,71, m (Zucker C-H's); 4,07—6,09, s und m (peptidische und aromatische CH's); 6,45—7,83, s und m (aromatische CH'S und peptidische NH's); 8,17—10,38 (peptidische NH's, phenolische OH's).

D) Retentionszeiten (R_t) von 1,27 und 1,32, bezogen auf Teicoplanin-A_2-Komponente 2 (R_t=20,3 min) bei Analyse durch Umkehrphasen-HPLC unter den folgenden Bedingungen:

Säule:
Ultrasphere® (5 µm) Altex (Beckman) 4,6 mm (Innendurchmesser)×250 mm

Vorsäule:
Brownlee® Labs RP 18 (5 µm)

Eluierungsmittel A:

CH_3CN	10%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	90%	auf pH 6,0

Eluierungsmittel B:

CH_3CN	70%	eingestellt
(2,5 g/l) $NaH_2PO_4 \cdot H_2O$	30%	auf pH 6,0

Eluierung:
linearer Gradient von 5% bis 60% des Eluierungsmittels B in Eluierungsmittel A, in 40 min

Fließgeschwindigkeit:
1,8 ml/min

UV-Detektor:
254 nm

Interner Standard:
Teicoplanin A_2-Komponente 2 (Gruppo Lepetit S.p.A.)

E) R_f-Wert, bezogen auf Teicoplanin A_2-Komponente 2, von 0,53 in dem folgenden chromatographischen System:

5 %iges (Gew./Vol.) wässeriges Na_2SO_4	70
Acetonitril	30

unter Verwendung von F_{254}-Merck-Platten mit silanisiertem Kieselsäuregel 60 (Schichtdicke 0,25 mm)

Sichtbarmachung:
—UV-Licht
—gelbe Farbe mit Pauly-Reagens, nämlich diazotierter Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99 (1953))
—Bioautographie unter Verwendung von B. subtilis ATCC 6633 auf Davis-Minimalmedium.

F) Molekulargewicht von etwa 1772, errechnet aus einem FAB-MS-Spektrum, das einen Haufen von Peaks mit dem intensivsten Peak bei 1776 zeigt. Die Arbeitsbedingungen der FAB-MS-Analyse waren die folgenden:

Instrument:
VG Mod ZAB SE, ausgestattet mit FAB gun Ion T ch

Bedingungen:
Positive FAB, Xe Beschl unigungsspannung, 8kV Matrix: Thioglycerin-Glyc rin 1/1 (Vol./V l.);

welches das Kultivieren des Stammes Actinomadura sp. ATCC 39727, oder einer Antibiotikum-A 40926-produzierenden Mutante oder Variante davon, unter submersen, aeroben Bedingungen in Gegenwart von assimilierbaren Quellen von Kohlenstoff, Stickstoff und anorganischen Salzen, das Gewinnen und das Isolieren des genannten Antibiotikums aus den Fermentationsbrühen und erforderlichenfalls das Umwandeln desselben in das gewünschte Salz, umfaßt.

2. Ein Verfahren wie in Anspruch 1, wobei der Stamm bei einer Temperatur zwischen 20°C und 40°C kultiviert wird.

3. Ein Verfahren wie in Anspruch 1, wobei die Temperatur zwischen 24°C und 35°C liegt.

4. Ein Verfahren wie in Anspruch 1, wobei die Gewinnung und Isolierung der antibiotischen Substanzen dadurch erzielt wird, daß die filtrierte Fermentationsbrühe einer Affinitätschromatographie an immobilisiertem D-Alanyl-Alanin, gefolgt von Verteilungs-, Umkehrphasenoder Ionenaustauscherchromatographie, unterworfen wird.

5. Ein Verfahren nach Anspruch 1, wobei die Gewinnung der antibiotischen Substanzen umfaßt:

a) das Unterwerfen der Fermentationsbrühe der Affinitätschromatographie an immobilisiertem D-Alanyl-D-Alanin

b) das rasche Neutralisieren der vereinigten, Antibiotikumhältigen, eluierten Fraktionen, und

c) gegebenenfalls das Isolieren der Antibiotikum-A 40926-Faktoren PA, PB, A, B und B_0 mittels Umkehrphasen-Flüssigkeitschromatographie an silanisiertem Kieselsäuregel.

6. Ein Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, ausgewählt aus Antibiotikum-A 40926-Komplex, Antibiotikum-A 40926-Faktor A, Faktor B und Faktor B_0, welches umfaßt:

a) das Basischstellen der Fermentationsmasse auf einen pH-Wert zwischen 8,5 und 10,5

b) das Filtrieren

c) das Ansäuren des klaren Filtrates auf einen pH-Wert von 2,5—4,5

d) das Filtrieren und das Verwerfen des Filtrates

e) das Suspendieren des Filterkuchens in Wasser und das Basischstellen desselben auf einen pH-Wert zwischen 8 und 9

f) das Unterwerfen des rohen Antibiotikum-A 40926-Komplexes, nach dessen Gewinnung durch Filtration, der Affinitätschromatographie an immobilisiertem D-Alanyl-D-Alanin

g) gegebenenfalls das Isolieren von Antibiotikum-A 40926-Faktor A und Faktor B mittels Verteilungs-, Umkehrphasen- oder Ionenaustauscherchromatographie, und

h) das Unterwerfen von Antibiotikum-A 40926-Faktor B einer weiteren Affinitätschromatographieverfahrensweise, falls Antibiotikum-A 40926-Faktor B_0 erwünscht ist.

7. Ein Verfahren nach Anspruch 6, wobei die chromatographische Methode Umkehrphasenchromatographie ist, und die stationäre Phase aus silanisiertem Kieselsäuregel und nicht-funktionalisierten Polystyrolharzen ausgewählt ist.

8. Ein Verfahren nach Anspruch 6, wobei die stationäre Phase silanisiertes Kieselsäuregel ist, das mit einer gepufferten Lösung bei einem pH-Wert zwischen 4 und 9 vor-äquilibriert worden ist, und wobei das Eluierungsmittel ein Lineargradientengemisch eines polaren, wassermischbaren Lösungsmittels in der gleichen, gepufferten Lösung ist.

9. Die Verwendung einer Verbindung von Anspruch 1 zur Herstellung eines Arzneimittels zum antibakteriellen Gebrauch.

Revendications pour les E'tats Contractants: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Substance antibiotique choisie parmi le complexe d'antibiotique A 40926, le facteur A de l'antibiotique A 40926, le facteur B de l'antibiotique A 40926, le facteur B_0 de l'antibiotique A 40926, le facteur PA de l'antibiotique A 40926, le facteur PB de l'antibiotique A 40926, un mélange de ceux-ci et les sels d'addition de ceux-ci, qui sont caractérisés comme suit:

Facteur A de l'antibiotique A 40926 sous forme non saline:

A) Spectre d'absorption ultraviolet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	281
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1 N	300
d) Méthan l	282
e) Tampon phosphate pH 9,0	282 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}):

3700—3100, 3100—2800 (nujol); 1655; 1620—1560; 1510; 1480—1410 (nujol); 1375 (nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (nujol).

C) Spectre de $RMN^{-1}H$ qui présente les groupes de signaux suivants (en ppm) à 270 MHz, enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm): δ 0,86 (triplets, 6H); 1,21 (≃11H); 1,43 (2H); 2,01 (2H); 2,31—2,34 (3H); 4—6,2 (≃16H); 6,2—8 (≃23H); 8,44, 9,22, 9,66 (bandes larges; protons mobiles).

2,5—4; interférence de pics de H_2O.

D) Temps de rétention (R_t) de 0,60 par rapport à la Testostérone, lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere ODS (5 μm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee Labs RP 18 (5 μm).

éluant A:

CH_3CN	10%	adjusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Testostérone (Roussel Uclaf)

E) Analyse élémentaire, après que l'échantillon a été séché au préalable à environ 140°C en atmosphère inerte (ΔP 4,6%), qui indique la composition approximative en pourcentage suivante (moyenne): carbon 55,82%; hydrogène 5,17%; azote 6,31%; chlore (total) 4,24%; chlore (ionique) 0,37%. Résidu minéral à 900°C dans l'air: 1,2%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS):eau 4:1 par titrage avec KOH après addition d'un excès de HCl, qui indique quatre fonctions ionisables ayant les pk_{MCS} suivants: 4,6, 5,6, 7,2, 9,2.

G) R_f de 0,24 et R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,70 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1716 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1717.

Facteur B de l'antibiotique A 40926 sous la forme non saline:

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1N	282
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1N	300
d) Tampon phosphate pH 9,0	283 300 (épaulement)
e) Méthanol	282

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^{1}H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

δ 0,85, (d, less CH_3 d'isopropyle); 1,15 (≃13H); 1,44 (≃2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (≃16H); 6,1—8 (≃23H); 8,52, 9,30, 9,68 (bandes larges; protons mobiles).

2,5—4: interférence de pics de H_2O.

D) Temps de rétention (R_t) de 1,22 et 1,27 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans le conditions suivantes:

colonne:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 μm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A.)

E) L'analyse élémentaire, après que l'échantillon a été séché au préalable à environ 140°C en atmosphère inerte (ΔP 9,6%), indique la composition approximative en pourcentage suivante (moyenne): carbon 54,09%; hydrogène 5,13%; azote 6,34%; chlore (total) 4,12%; chlore (ionique) 0,39%. Résidu minéral à 900°C dans l'air: 5%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS):eau 4:1 par titrage avec KOH après addition d'un excès de HCl (pH 2,7), qui indique quatre fonctions i nisables ayant les pk_{MCS} suivants: 4,5, 5,6, 7,2, 9,2.

G) R_f de 0,21 et R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,53 dans le système chr matographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:

—lumière U.V.

—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))

—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1730 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1731.

Facteur B_0 de l'antibiotique A 40926 sous la forme non saline:

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1 N	300
d) Tampon phosphate pH 9,0	283 300 (épaulement)
e) Méthanol	282

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3800, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) Spectre de RMN-^1H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^1H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

δ 0,85 (d, les CH_3 d'isopropyle); 1,15 (≃13H); 1,44 (≃2H); 2,02 (2H); 2,32—2,35 (3H), 4—6,1 (≃16H); 6,1—8 (≃23H); 8,52, 9,30, 9,68 (bandes larges; protons mobiles).

2,5—4: interférence de pics de H_2O.

D) Temps de rétention R_t) de 1,22 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:

Ultrasphere® ODS (5 μm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:

Brownlee® Labs RP 18 (5 μm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:

gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:

1,8 ml/min

détect ur U.V.:

254 nm

Etal n interne:

Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A.)

E) L'analyse élémentaire, après que l'échantillon a été séché au préalable à environ 140°C en atmosph`re inerte (ΔP 9,6%), indique la composition approximative en pourcentage suivante (moyenne):

carbon 54,09%; hydrogène 5,13%; azote 6,34%; chlore (total) 4,12%; chlore (ionique) 0,39%. Résidue minéral à 900°C dans l'air: 5%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS):eau 4:1 après titrage avec KOH après addition d'un excès de HCl qui indique quatre fonctions ionisables ayant les pk_{MCS} suivants: 4,5, 5,6, 7,2, 9,2.

G) R_f de 0,21 et R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,53 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:

—lumière U.V.

—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))

—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1730 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1731.

Facteur PA de l'antibiotique A 40926 sous la forme non saline:

A) spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Hydroxyde de potassium 0,1 N	300
c) Tampon phosphate pH 7,38	282 300 (épaulement)
d) Tampon phosphate pH 9,0	283 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}):

3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (nujol); 1375 (nujol); 1260; 1250—950; 845; 805; 720 (nujol).

C) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^{1}H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadéutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

0,86, d's(CH_3); 1,15—1,22, m $(CH_2)_n$; 1,41, m (CH_2); 2,01, s (CH_3); 2,01, m (CH_2); 2,28, s (N-CH_3); 4,26—5,96, br (groupes CH peptidiques et aromatiques); 6,33—7,73 br (groupes CH aromatiques et groupes NH peptidiques).

br = large
d = doublet
dd = doublet de doublets
m = multiplet
s = singulet
t = triplet

D) Temps de rétention (R_t) de 1,15 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5µm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A.)

E) R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,62 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche, 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

F) MM d'environ 1758 déduite d'un spectre de FAB-SM présentant un amas de pics ayant le pic le plus intense à 1761. Les conditions opératoires de l'analyse par FAB-SM ont été les suivantes:

Instrument:
VG Mod ZAB SE équipé d'un canon FAB Ion Tech

Conditions:
FAB positif, Xe Tension d'accélération, 8KV Matrice: Thiogycérol-glycérol 1/1 (v/v)

Facteur PB de l'antibiotique A 40926 sous la forme non saline.

A) spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Hydroxyde de potassium 0,1 N	300
c) Tampon phosphate pH 7,38	282 300 (épaulement)
d) Tampon phosphate pH 9,0	282 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}):
3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (nujol); 1375 (nujol); 1320—1270; 1230—1190; 1150; 1120—920; 845; 810; 720 (nujol).

C.1) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle), en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm) multiplicité; (attribution):
0,84 d (groupes CH_3 de l'isopropyle); 1,17, m $(CH_2)_n$; 1,43, m (CH_2); 1,99, s (CH_3); 2,01, m (CH_2); 2,31, s $(N\text{-}CH_3)$; 2,79, dd (C-H); 3,70, m (C-H); 4,06—6,02, br (gr upes CH p ptidiques et aromatiqu s); 6,45—7,74 br (groupes CH aromatiques et groupes NH peptidiques); 8,19—9,99, br (groupes NH peptidiques et groupes OH phénoliques).

C.2) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) à 270 MHz enregistrés

dans du DMSO d_6 plus CF_3COOD en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm) multiplicité; (attribution):

0,84, d (gr upes CH_3 de l'isopropyle); 1,13, m $(CH_2)_n$; 1,40, m (CH_2); 1,98, s (CH_3); 2,00, m (CH_2); 2,92, dd (C-H); 3,29—3,71, m (groupes CH sucr); 4,07—6,09, s et m (groupes CH peptidiques et aromatiques); 6,45—7,83, s et m (groupes CH aromatiques et groupes NH peptidiques); 8,17—10,38 (groupes NH peptidiques et groupes OH phénoliques).

D) Temps de rétention (R_t) de 1,27 et 1,32 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 µm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A)

E) R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,53 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 (F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

F) MM d'environ 1772 déduite d'un spectre de FAB-SM présentant un amas de pics ayant le pic le plus intense à 1776. Les conditions opératoires de l'analyse par FAB-SM ont été les suivantes:

Instrument:
VG Mod ZAB SE équipé d'un canon FAB Ion Tech

Conditions:
FAB positive, Xe Tension d'accérlation, 8KV Matrice: Thioglycérol-glycérol 1/1 (v/v)

2. Procédé de préparation d'un composé selon la revendication 1, qui comprend la culture de la souche *Actinomadura sp.* ATCC 39727, ou d'un mutant ou variant de celle-ci produisant l'antibiotique A 40926 dans des conditions aérobies immergées, en présence de sources assimilables de carbone, d'azote et de sels minéraux, la récupération et l'isolement de cet antibiotique des bouillons de fermentation et sa transformation en le sel désiré, si nécessaire.

3. Procédé sel n la revendication 2, dans lequel la souche st cultivée à une température comprise en 20°C et 40°C.

4. Procédé selon la revendication 3, dans lequel la température est comprise entre 24°C et 35°C.

5. Procédé selon la revendication 2, dans lequel la récupération et l'isolement des substances antibiotiques sont obtenus en soumettant le bouillon de fermentation filtré à une chromatographie d'affinit' sur D-alanyl-D-alanine immobilisée, suivie d'une chromatographie de partage, en phase inversée ou par échange d'ions.

6. Procédé selon la revendication 2, dans lequel la récupération des substances antibiotiques comprend:

a) le fait de soumettre le bouillon de fermentation à une chromatographie d'affinité sur de la D-Alanyl-D-alanine immobilisée

b) la neutralisation rapide des fractions éluées contenant l'antibiotique mélangé et

c) l'isolement si on le désire des factuers PA, PB, A, B et B_0 de l'antibiotiques A 40926 au moyen d'une chromatographie liquide en phase inversée sur un gel de silice silanisé.

7. Procédé selon la revendication 2, pour préparer un composé choisi parmi un complexe d'antibiotique A 40926, le facteur A, le facteur B et le facteur B_0 de l'antibiotique A 40926, qui comprend:

a) l'alcalinisation de la masse de fermentation à un pH compris entre 8,5 et 10,5

b) la filtration

c) l'acidification du filtrat limpide à pH 2,5—4,5

d) la filtration et l'évacuation du filtrat

e) la mise en suspension du gâteau de filtration dans de l'eau et son alcalinisation à un pH compris entre 8 et 9

f) après récupération du complexe brut d'antibiotique A 40926 par filtration, le fait de la soumettre à une chromatographie d'affinité sur D-alanyl-D-alanine immobilisée

g) l'isolement si on le désire du facteur A et du facteur B de l'antibiotique A 40926 par chromatographie de partage, en phase inversée ou par échange d'ions, et

h) le fait de soumettre le facteur B de l'antibiotique A 40926 à une opération de chromatographie d'affinité supplémentaire lorsqu'on désire le facteur B_0 de l'antibiotique A 40926.

8. Procédé selon la revendication 7, dans lequel la technique chromatographie est une chromatographie en phase inversée et la phase stationnaire est choisie parmi un gel de silice silanisé et des résines de polystyrène non fonctionnalisées.

9. Procédé selon la revendication 7, dans lequel la phase stationnaire est un gel de silice silanisé pré-équilibré avec une solution tamponnée à un pH compris entre 4 et 9 et l'éluant est un mélange à gradient linéaire d'un solvant polaire miscible à l'eau dans la même solution tamponnée.

10. Composé selon la revendication 1, pour l'utilisation comme médicament.

11. Composition pharmaceutique qui comprend un composé de la revendication 1 en mélange avec un véhicule pharmaceutiquement acceptable.

12. La souche *Actinomadura* sp. ATCC 39727.

13. Culture biologiquement pure de la souche *Actinomadura sp.* ATCC 39727 ou d'un mutant ou variant de celle-ci produisant l'antibiotique A 40926, capable de produire un composé de la revendication 1 lorsqu'elle est cultivée dans des conditions aérobies immergées en présence de sources assimilables de carbone, d'azote et de sels minéraux.

Revendications pour l'Etat Contractant: AT

1. Procédé pour préparer une substance antibiotique choisie parmie le complexe d'antibiotique A 40926, le facteur A de l'antibiotique A 40926, le facteur B de l'antibiotique A 40926, le facteur B_0 de l'antibiotique A 40926, le facteur PA de l'antibiotique A 40926, le facteur PB de l'antibiotique A 40926, un mélange de ceux-ci et les sels d'addition de ceux-ci, qui sont caractérisé comme suit:

Facteur A de l'antibiotique A 40926 sous forme non saline:

A) Spectre d'absorption ultraviolet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	281
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1 N	300
d) Méthanol	282
) Tampon phosphate pH 9,0	282 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3100, 3100—2800 (nujol); 1655; 1620—1560; 1510; 1480—1410 (nujol); 1375 (nujol); 1320—1250; 1250—1190; 1100—950; 845; 810; 720 (nujol).

C) Spectre de RMN-^1H qui présente les groupes de signaux suivants (en ppm) à 270 MHz, enregistrés dans du DMSO d_6 (sulfoxyde d'hexadéutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

δ 0,86 (triplets, 6H); 1,21 (≃11H); 1,43 (2H); 2,01 (2H); 2,31—2,34 (3H); 4—6,2 (≃16H); 6,2—8 (≃23H); 8,44, 9,22, 9,66 (bandes larges; protons mobiles).

2,5—4: interférence de pics de H_2O.

D) Temps de rétention (R_t de 0,60 par rapport à la Testostérone, lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere ODS (5 µm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee Labs RP 18 (5 µm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V:
254 nm

Etalon interne:
Testostérone (Roussel Uclaf)

E) Analyse élémentaire, après qui l'échantillon a été séché au préalable à environ 140°C en atmosphère inerte (ΔP 4,6%), qui indique la composition approximative en pourcentage suivante (moyenne): carbon 55,82%; hydrogène 5,17%; azote 6,31%; chlore (total) 4,24%; chlore (ionique) 0,37%. Résidu minéral à 900°C dans l'air: 1,2%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS): eau 4:1 par titrage avec KOH après addition d'un excès de HCl, qui indique quatre fonctions ionisables ayant les pk_{MCS} suivants: 4,6, 5,6, 7,2, 9,2.

G) R_f de 0,24 et R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,70 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1716 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1717.

Facteur B de l'antibiotique A 40926 sous la forme non saline:

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1 N	300
d) Tampon phosphate pH 9,0	283 300 (épaulement)
e) Méthanol	282

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^{1}H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadéutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

δ 0,85 (d, les CH_3 d'isopropyle); 1,15 (≃13H); 1,44 (≃2H); 2,02 (2H); 2,32—2,35 (3H); 4,—6,1 (≃16H); 6,1—8 (≃23H); 8,52, 9,30, 9,68 (bandes larges; protons mobiles).

2,5—4: interférence de pics de H_2O.

D) Temps de rétention (R_t) de 1,22 et 1,27 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 µm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A)

E) L'analyse élémentaire, après que l'échantillon a été séché au préalable à environ 140°C en atmosphère inrete (ΔP 9,6%), indique la composition approximative en pourcentage suivante (moyenne):

carbone 54,09%; hydrogène 5,13%; azote 6,34%; chlore (total) 4,12%; chlore (ionique) 0,39%. Résidu minéral à 900°C dans l'air: 5%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS):eau 4:1 par titrage avec KOH après addition d'un excès de HCl (pH 2,7), qui indiqu quatre fonctions ionisables ayant les pk_{MCS} suivants: 4,5, 5,6, 7,2, 9,2.

G) R_f de 0,21 et R_f par rapport au constituant 2 de la T'icoplanine A_2 de 0,53 dans le syst'me chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20,* 171 (1965), Z. Physiol. Chem. *292,* 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1730 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1731.

Facteur B_0 de l'antibiotique A 40926 sous la forme non saline:

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Tampon phosphate pH 7,38	281 300 (épaulement)
c) Hydroxyde de sodium ou de potassium 0,1 N	300
d) Tampon phosphate pH 9,0	283 300 (épaulement)
e) Méthanol	282

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3080, 3080—2700 (nujol); 1720—1625; 1625—1560; 1505; 1460 (nujol); 1375 (nujol); 1295; 1230; 1210; 1150; 1100—1040; 1030; 1015; 970; 890; 840; 810; 720 (nujol).

C) Spectre de RMN-^1H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^1H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

δ 0,85 (d, les CH_3 d'isopropyle); 1,15 (≃13H); 1,44 (≃2H); 2,02 (2H); 2,32—2,35 (3H); 4—6,1 (≃16H); 6,1—8 (≃23H); 8,52, 9,30, 9,68 (bandes larges; protons mobiles).

2,54—4: interférence de pics de H_2O.

D) Temps de rétention (R_t) de 1,22 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 μm)

éluant A:

CH_3CN	10%	} ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	} pH 6,0

éluant B:

CH_3CN	70%	} ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	} pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
C nstituant 2 de la Téic planine A_2 (Gruppo Lepetit S.p.A.)

E) L'analyse élémentaire, après que l'échantillon a été séché au préalable à environ 140°C en atmosphère inerte (ΔP 9,6%), indique la composition approximative en pourcentage suivante (moyenne): carbone 54,09%; hydr gène 5,13%; azote 6,34%; chlore (total) 4,12%; chlore (ionique) 0,39%. Résidu minéral à 900°C dans l'air: 5%.

F) Profil de titrage acide-base dans le 2-méthoxyéthanol (MCS):eau 4:1 après titrage avec KOH aprés addition d'un excès de HCl qui indique quatre fonctions ionisables ayant les pk_{MCS} suivants: 4,5, 5,6, 7,2, 9,2.

G) R_f de 0,21 et R_t par rapport au constituant 2 de la Téicoplanine A_2 de 0,53 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:

—lumière U.V.

—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))

—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

H) MM d'environ 1730 déduite d'un spectre de FAB-SM présentant le pic $M+H^{\oplus}$ à 1731.

Facteur PA de l'antibiotique A 40926 sous la forme non saline:

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Hydroxyde de potassium 0,1 N	300
c) Tampon phosphate pH 7,38	282 300 (épaulement)
d) Tampon phosphate pH 9,0	283 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}): 3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1550; 1505; 1460 (nujol); 1375 (nujol); 1260; 1250—950; 845; 805; 720 (nujol).

C) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) dans la RMN-^{1}H à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadeutérodiméthyle) en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm):

0,86, d's(CH_3); 1,15—1,22, m $(CH_2)_n$; 1,41, m (CH_2); 2,01, s (CH_3); 2,01, m (CH_2); 2,28, s (N-CH_3); 4,26—5,96, br (groupes CH peptidiques et aromatiques); 6,33—7,73 br (groupes CH aromatiques et groupes NH peptidiques).

br = large
d = doublet
dd = doublet de doublets
m = multiplet
s = singulet
t = triplet

D) Temps de rétention (R_t) de 1,15 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 μm) Altex (Beckman) 4,6 mm (d.i)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 μm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A)

E) R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,62 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautographie en utilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

F) MM d'environ 1758 déduite d'un spectre de FAB-SM présentant un amas de pics ayant le pic le plus intense à 1761. Les conditions opératoires de l'analyse par FAB-SM ont été les suivantes:

Instrument:
VG Mod ZAB SE équipé d'un canon FAB Ion Tech

Conditions:
FAB positif, Xe Tension d'accélération, 8KV Matrice: Thioglycérol-glycérol 1/1 (v/v)

Facteur PB de l'antibiotique A 40926 sous la forme non saline.

A) Spectre d'absorption ultra-violet qui présente les maxima d'absorption suivants:

	λ max (nm)
a) HCl 0,1 N	282
b) Hydroxyde de potassium 0,1 N	300
c) Tampon phosphate pH 7,38	282 300 (épaulement)
d) Tampon phosphate pH 9,0	282 300 (épaulement)

B) Spectre d'absorption infrarouge qui présente les maxima d'absorption suivants (cm^{-1}):
3700—3100, 3000—2800 (nujol); 1760—1710; 1655; 1620—1560; 1605; 1480—1420 (nujol); 1375 (nujol); 1302—1270; 1230—1190; 1150; 1120—920; 810; 720 (nujol).

C.1) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) à 270 MHz enregistrés dans du DMSO d_6 (sulfoxyde d'hexadéutérodiméthyle), en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm) multiplicité; (attribution):
0,84 d (groupes CH_3 de l'isopropyle); 1,17, m $(CH_2)_n$; 1,43, m (CH_2); 1,99, s (CH_3); 2,01, m (CH_2); 2,31, s (N-CH_3); 2,79, dd (C-H); 3,70, m (C-H); 4,06—6,02, br (gr upes CH peptidiques et aromatiques); 6,45—7,74 br (group s CH aromatiques et groupes NH peptidiques); 8,19—9,99, br (groupes NH peptidiques et groupes OH phénoliques).

C.2) Spectre de RMN-^{1}H qui présente les groupes de signaux suivants (en ppm) à 270 MHz enregistrés

dans du DMSO d_6 plus CF_3COOD en utilisant du TMS comme étalon interne (0,00 ppm), (δ=ppm) multiplicité; (attribution);

0,84, d (gr upes CH_3 de l'is propyle); 1,13, m $(CH_2)_n$; 1,40, m (CH_2); 1,98, s $(CH)_3$; 2,00, m (CH_2); 2,92, dd (C-H); 3,29—3,71, m (groupes CH sucre); 4,07—6,09, s et m (groupes CH peptidiques et ar matiques); 6,45—7,83, s t m (groupes CH aromatiques et groupes NH peptidiques); 8,17—10,38 (groupes NH peptidiques et groupes OH phénoliques).

D) Temps de rétention (R_t) de 1,27 et 1,32 par rapport au constituant 2 de la Téicoplanine A_2 (R_t=20,3 min) lorsqu'il est analysé par HPLC en phase inversée dans les conditions suivantes:

colonne:
Ultrasphere® ODS (5 µm) Altex (Beckman) 4,6 mm (d.i.)×250 mm

pré-colonne:
Brownlee® Labs RP 18 (5 µm)

éluant A:

CH_3CN	10%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	90%	pH 6,0

éluant B:

CH_3CN	70%	ajusté à
$NaH_2PO_4 \cdot H_2O$ (2,5 g/l)	30%	pH 6,0

élution:
gradient linéaire de 5% à 60% de l'éluant B dans l'éluant A, en 40 min

débit:
1,8 ml/min

détecteur U.V.:
254 nm

Etalon interne:
Constituant 2 de la Téicoplanine A_2 (Gruppo Lepetit S.p.A)

E) R_f par rapport au constituant 2 de la Téicoplanine A_2 de 0,53 dans le système chromatographique suivant:

Na_2SO_4 aqueux à 5% (P/V)	70
Acétonitrile	30

en utilisant des plaques de gel de silice silanisé 60 F_{254} Merck (épaisseur de couche 0,25 mm)

Visualisation:
—lumière U.V.
—coloration jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. *20*, 171 (1965), Z. Physiol. Chem. *292*, 99, (1953))
—Bioautographie en untilisant *B. subtilis* ATCC 6633 sur du milieu minimum de Davis.

F) MM d'environ 1772 déduite d'un spectre de FAB-SM présentant un amas de pics ayant le pic le plus intense à 1776. Les conditions opératoires de l'analyse par FAB-SM ont été les suivantes:

Instrument:
VG Mod ZAB SE équipé d'un canon FAB Ion Tech

Conditions:
FAB positif, Xe Tension d'accélération, 8KV Matrice: Thioglycérol-glycérol 1/1 (v/v),

qui comprend la culture de la souche *Actinomadura sp.* ATCC 39727, ou d'un mutant ou variant de celle-ci produisant l'antibiotique A 40926 dans des conditions aérobies immergées en présence de sources assimilables de carbone, d'azote et de sels minéraux, la récupération et l'isolement de cet antibiotiques des b uillons de fermentation ét sa transf rmation et le s l désiré, si nécessaire.

2. Procédé selon la revendicati n 1, dans lequel la souche est cultivée à une température comprise n 20°C et 40°C.

3. Procédé sel n la revendication 2, dans lequel la températur st comprise entre 24°C et 35°C.

4. Procédé s lon la r vendication 1, dans lequel la récupération et l'isolement d s substances

antibiotiques sont obtenus en soumettant le bouillon de fermentation filtré à une chromatographie d'affinité sur D-alanyl-D-alanine immobilisée, suivie d'une chromatographie de partage, en phase inversée ou par échange d'ions.

5. Procédé selon la revendication 1, dans lequel la récupération des substances antibiotiques comprend:

a) le fait de soumettre le bouillon de fermentation à une chromatographie d'affinité sur de la D-alanyl-D-alanine immobilisée

b) la neutralisation rapide des fractions éluées contenant l'antibiotique mélangé et

c) l'isolement si on le désire des facteurs PA, PB, A, B et B_0 de l'antibiotique A 40926 au moyen d'une chromatographie liquide en phase inversée sur un gel de silice silanisé.

6. Procédé selon la revendication 1, pour préparer un composé choisi parmi un complexe d'antibiotique A 40926, le facteur A, le facteur B et le facteur B_0 de l'antibiotique A 40926, qui comprend:

a) l'alcalinisation de la masse de fermentation à un pH compris entre 8,5 et 10,5

b) la filtration

c) l'acidification du filtrat limpide à pH 2,5—4,5

d) la filtration et l'évacuation du filtrat

e) la mise en suspension du gâteau de filtration dans de l'eau et son alcalinisation à un pH compris entre 8 et 9

f) après récupération du complexe brut d'antibiotique A 40926 par filtration, le fait de le soumettre à une chromatographie d'affinité sur D-alanyl-D-alanine immobilisée

g) l'isolement si on le désire du facteur A et du facteur B de l'antibiotique A 40926 par chromatographie de partage, en phase inversée ou par échange d'ions, et

h) le fait de soumettre le facteur B de l'antibiotique A 40926 à une opération de chromatographie d'affinité supplémentaire lorsqu'on désire le facteur B_0 de l'antibiotique A 40926.

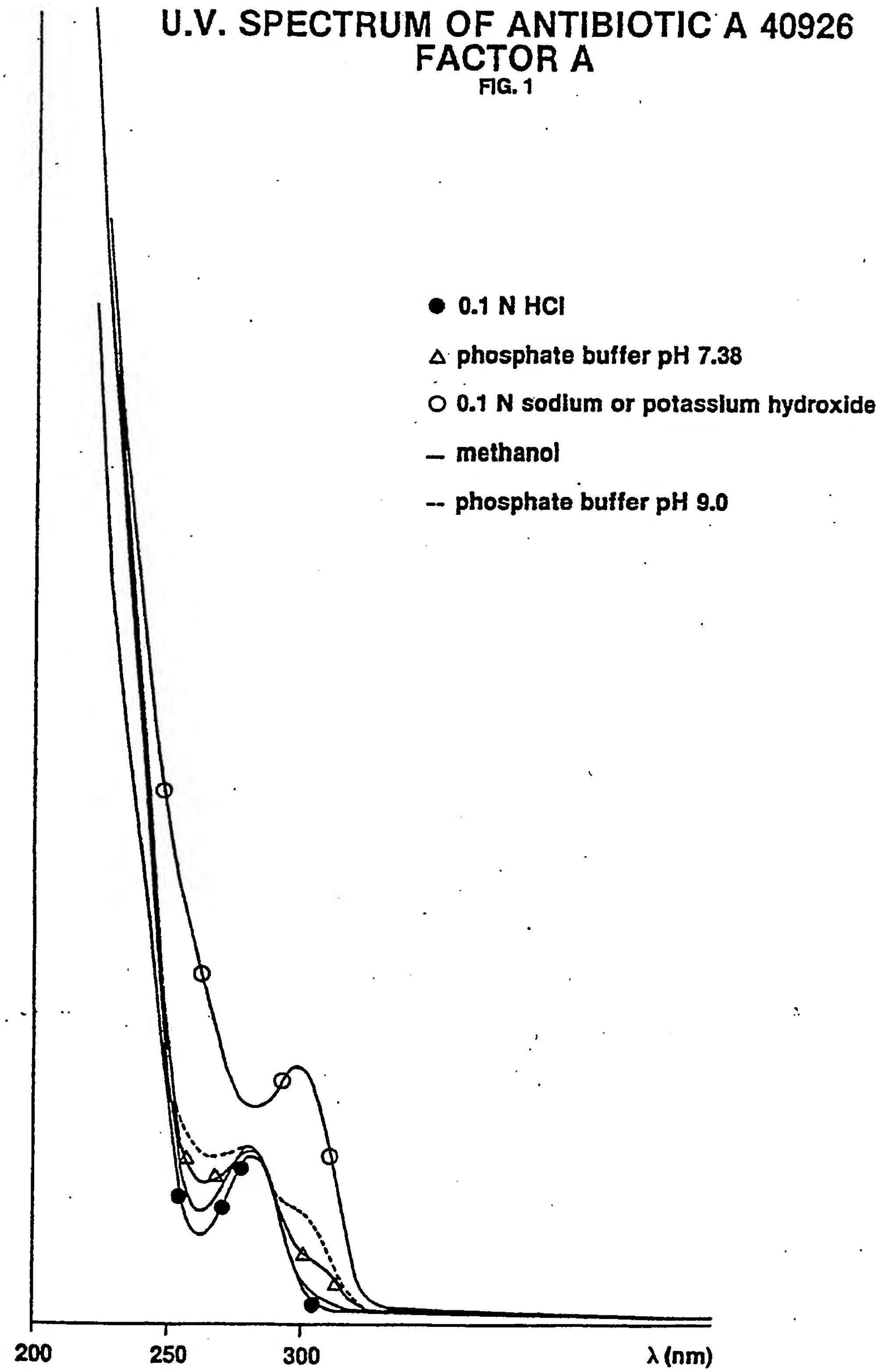
7. Procédé selon la revendication 6, dans lequel la technique chromatographique est une chromatographie en phase inversée et la phase stationnaire est choisie parmi un gel de silice silanisé et des résines de polystyrène non fonctionnalisées.

8. Procédé selon la revendication 6, dans lequel la phase stationnaire est un gel de silice silanisé pré-équilibré avec une solution tamponnée à un pH compris entre 4 et 9 et l'éluant est un mélange à gradient linéaire d'un solvant polaire miscible à l'eau dans la même solution tamponnée.

9. Utilisation d'un composé selon la revendication 1, pour préparer un médicament à usage antibactérien.

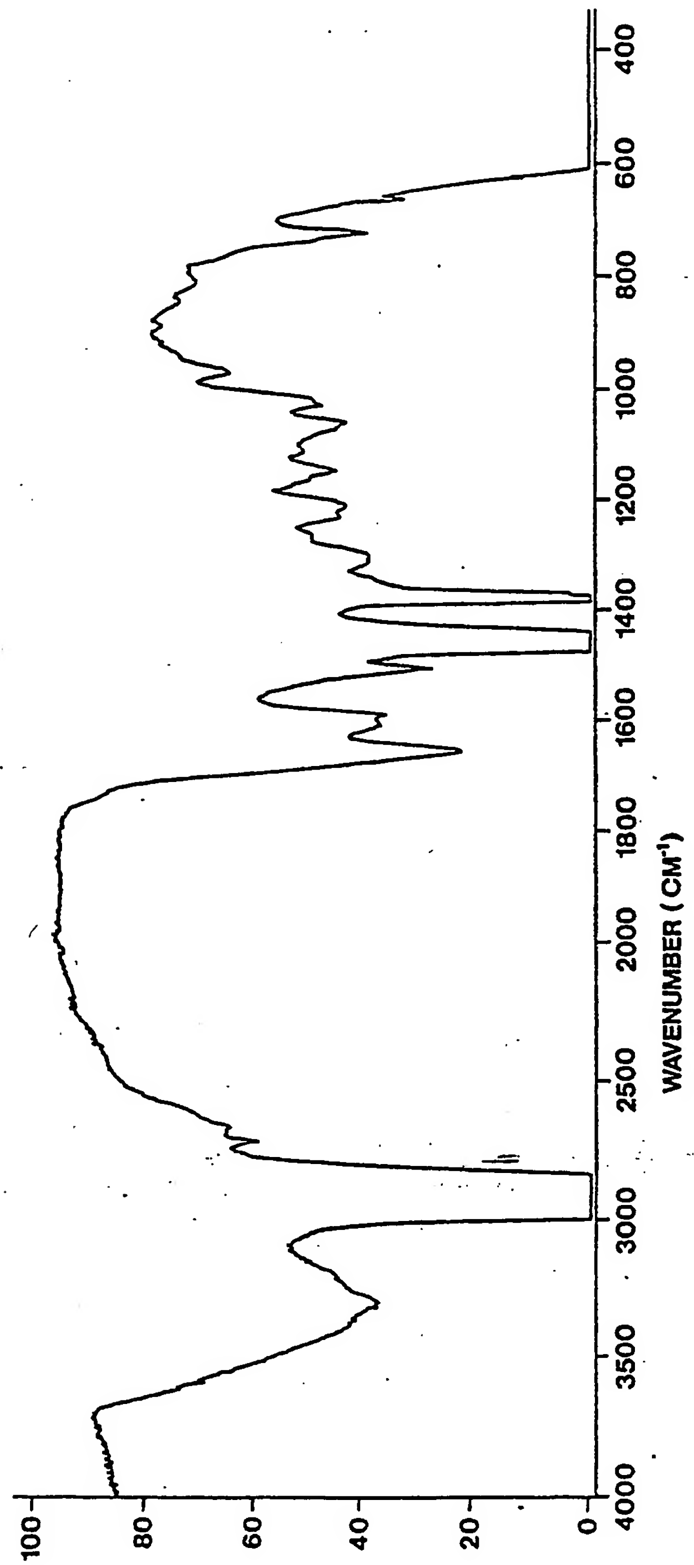
U.V. SPECTRUM OF ANTIBIOTIC A 40926
FACTOR A

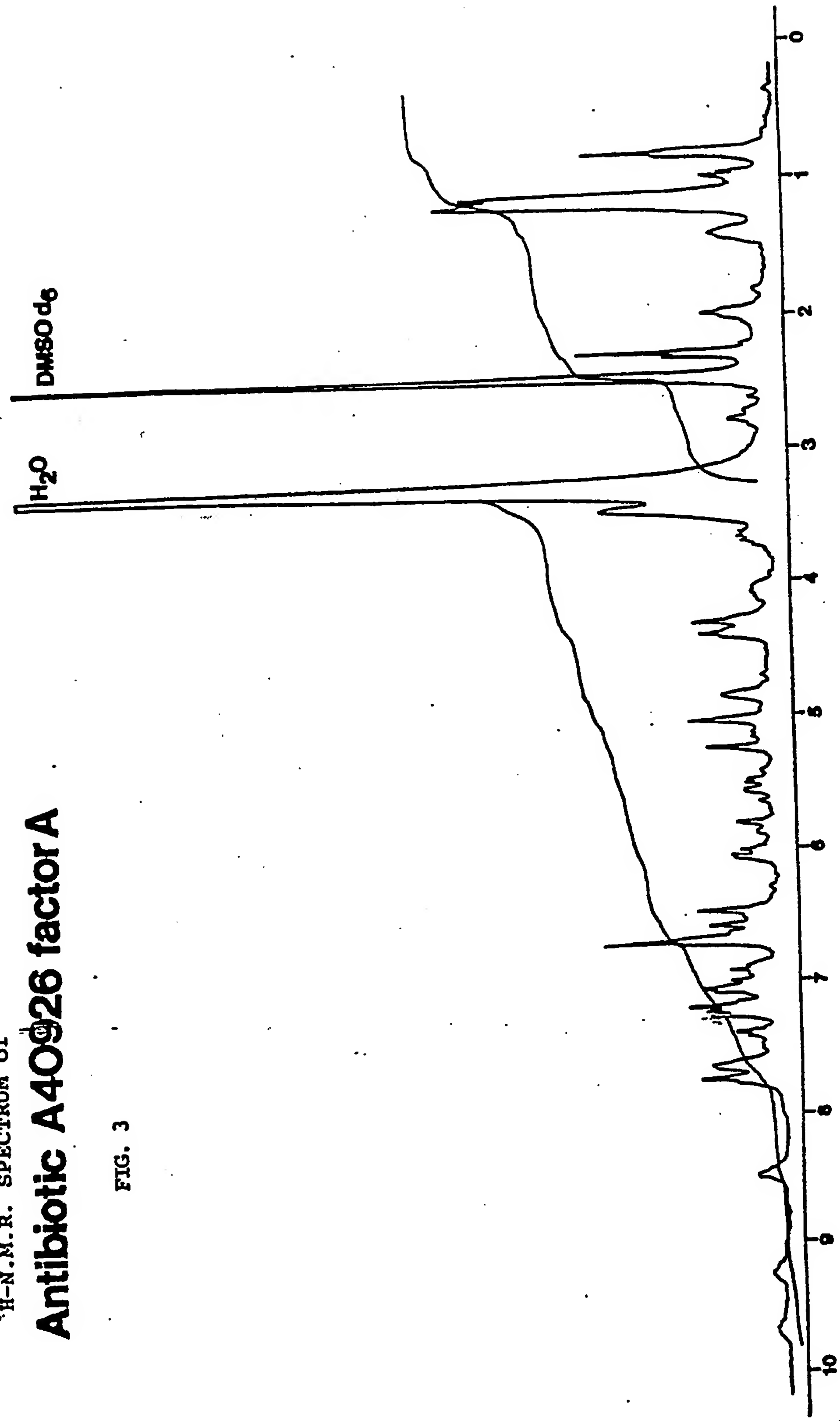
FIG. 1



I.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR A

FIG. 2





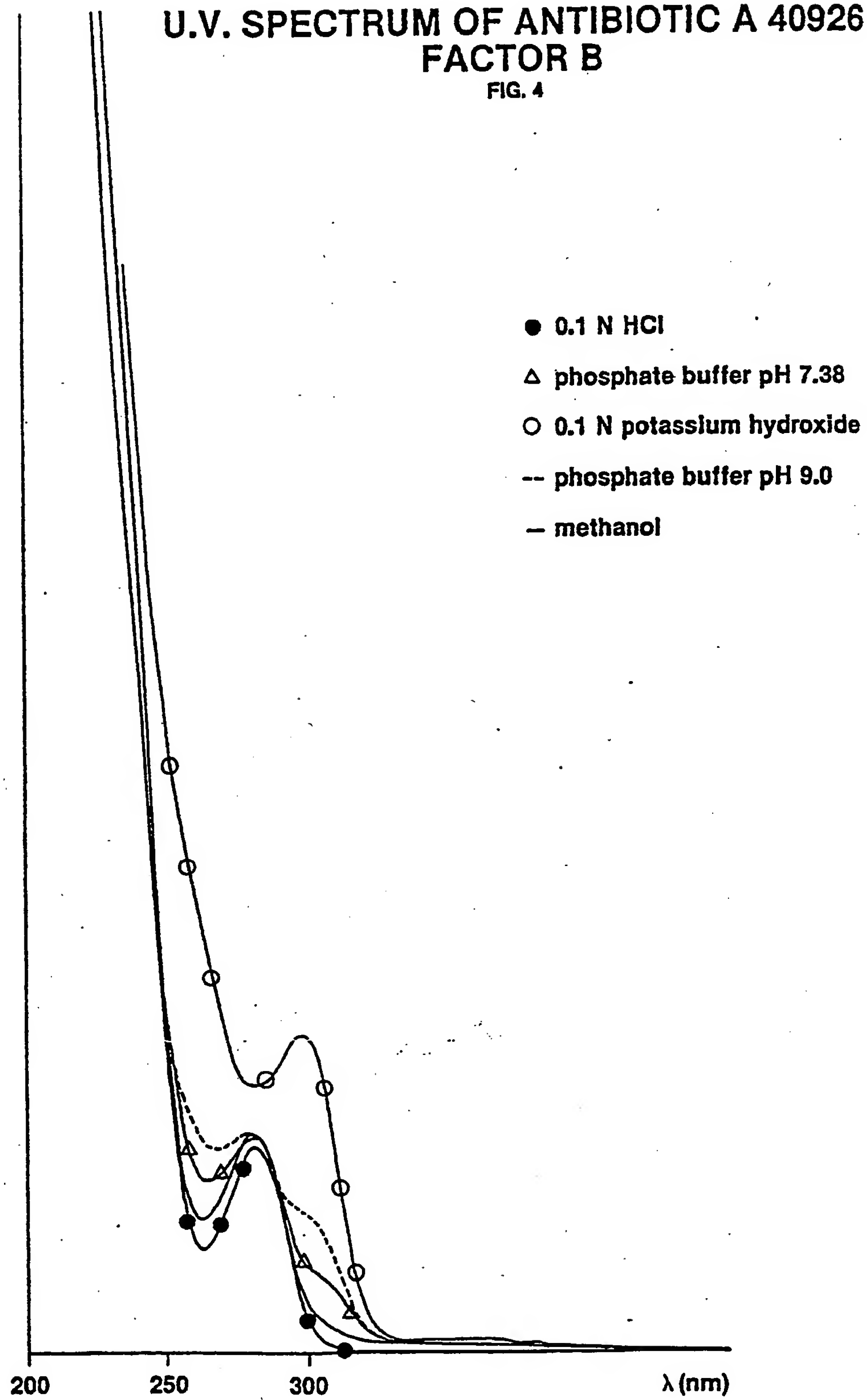
^{1}H-N.M.R. SPECTRUM of

Antibiotic A40926 factor A

FIG. 3

U.V. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR B

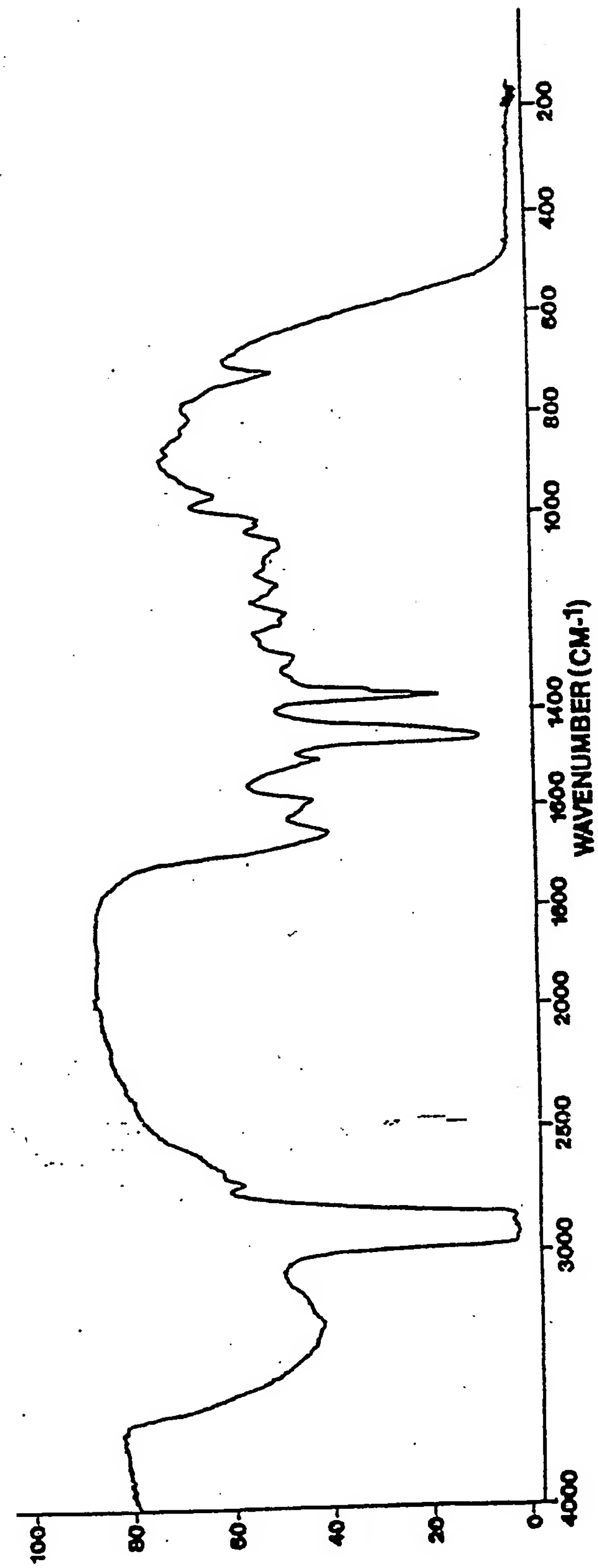
FIG. 4



I.R. SPECTRUM OF

A40926 factor B

FIG. 5



^{1}H-N.M.R. SPECTRUM OF

Antibiotic A40926 factor B

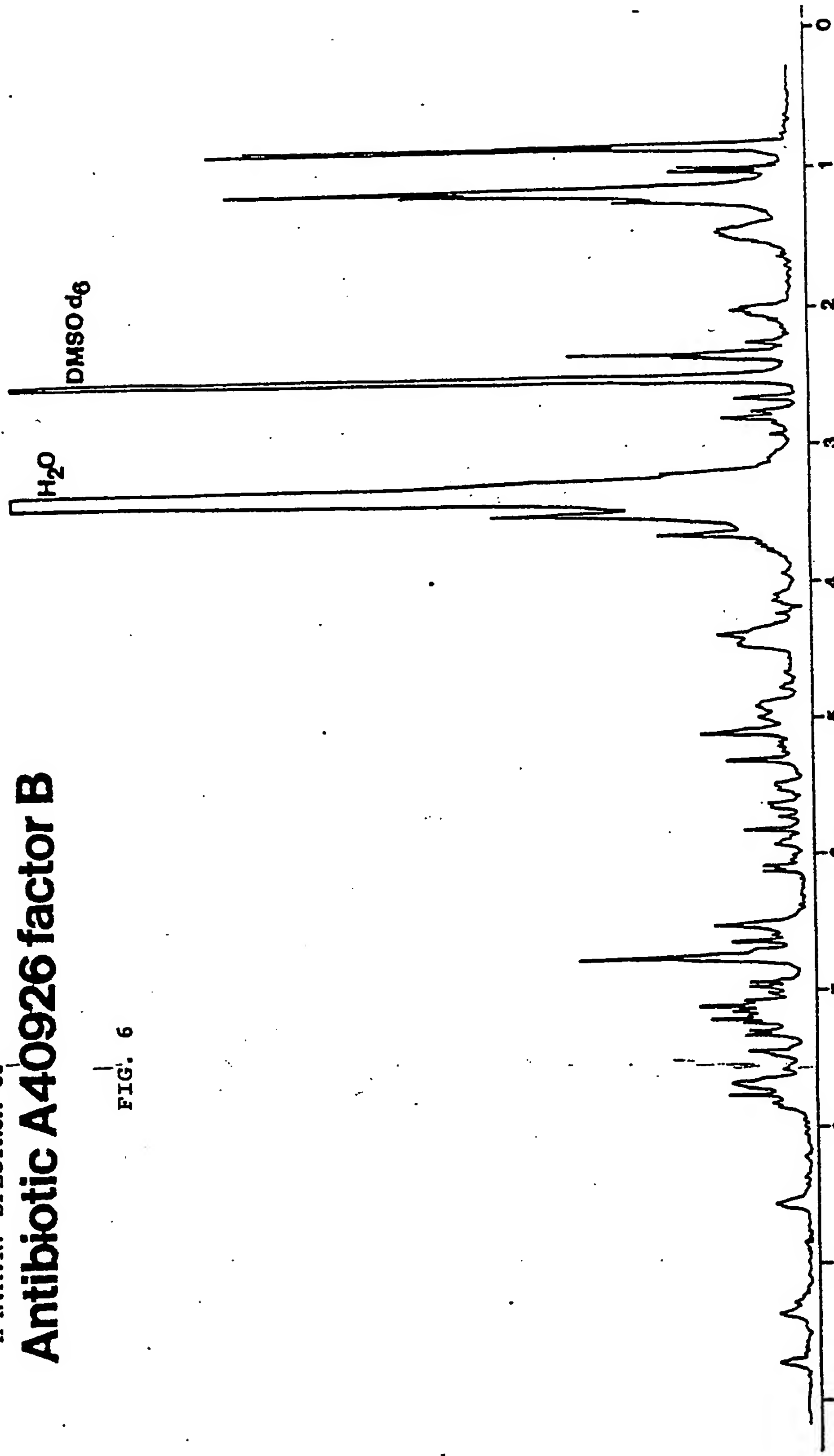
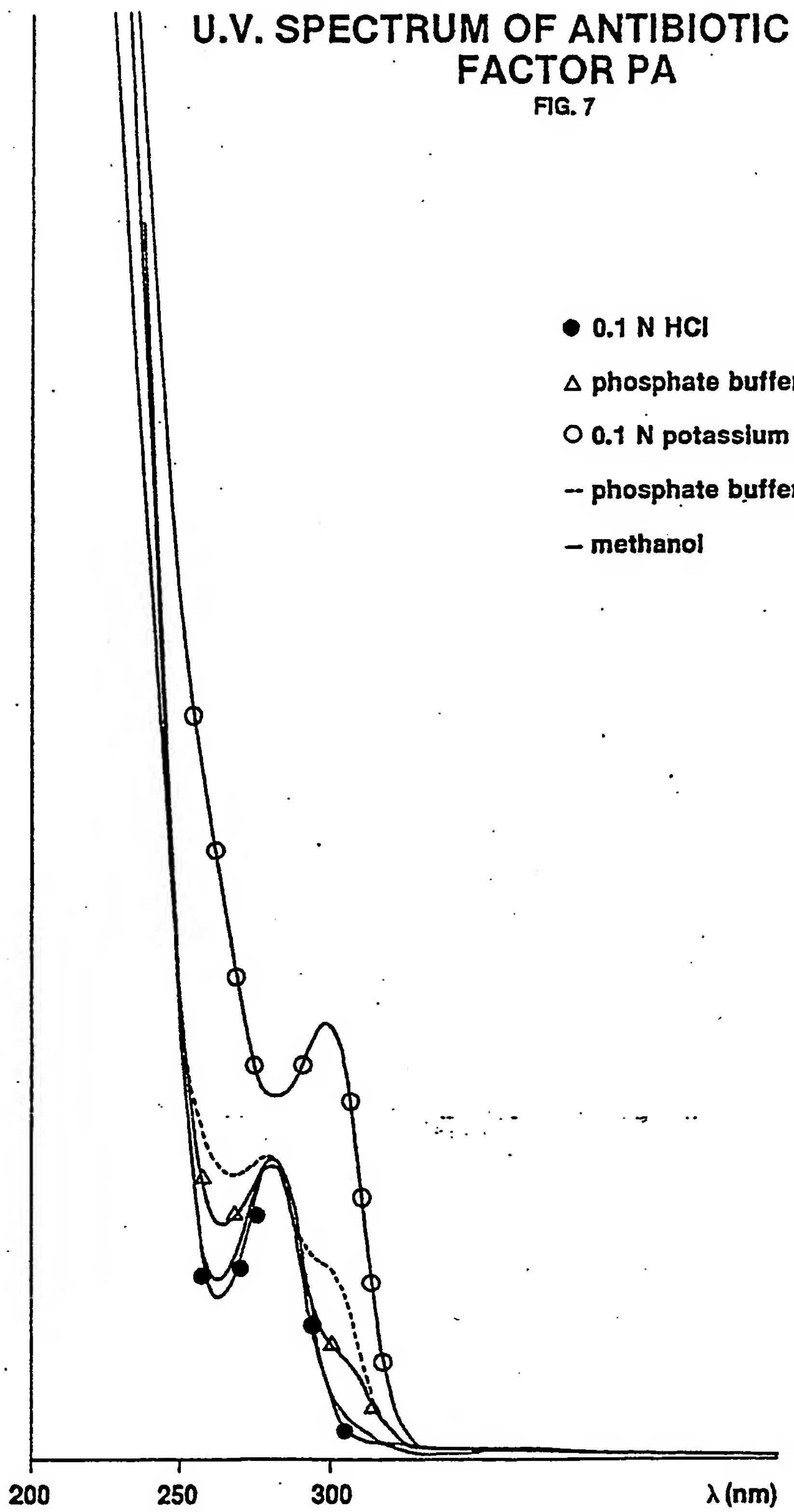


FIG. 6

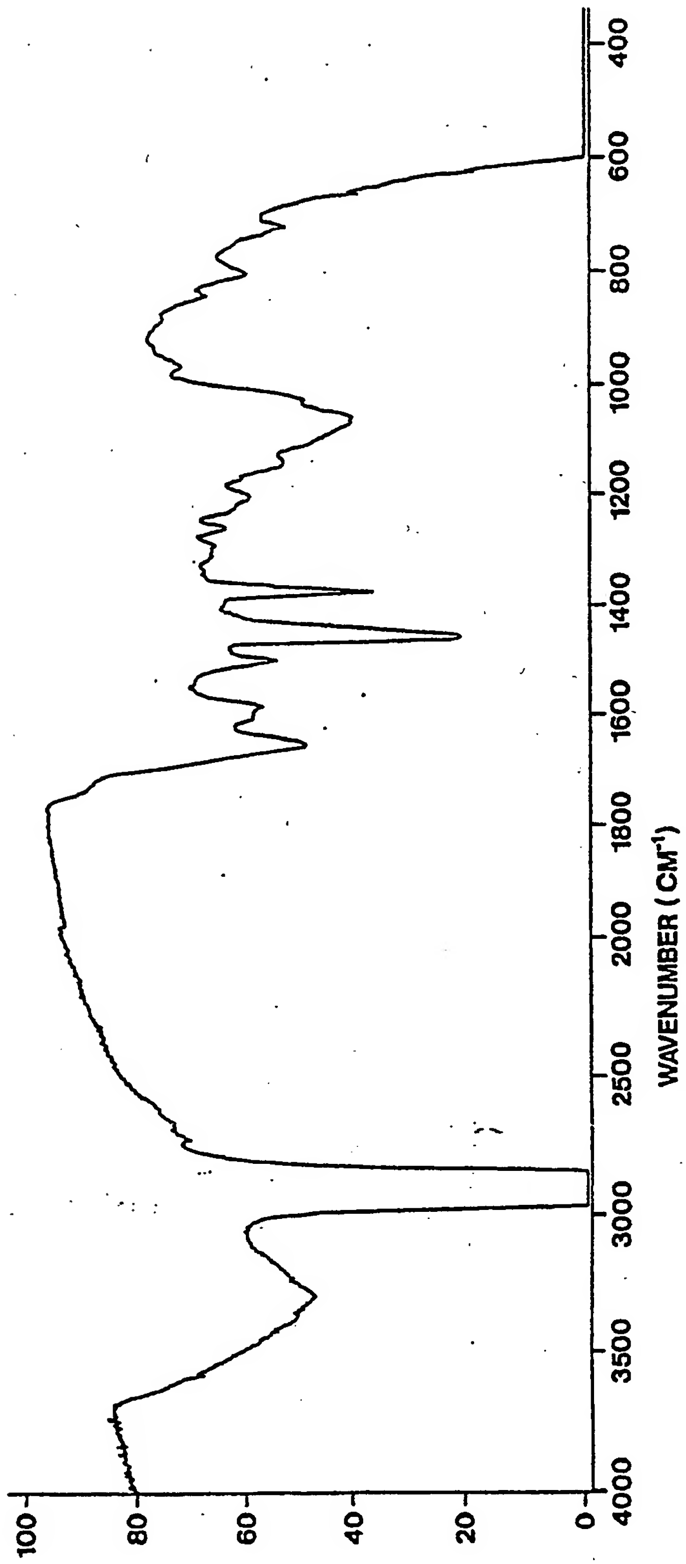
U.V. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PA

FIG. 7



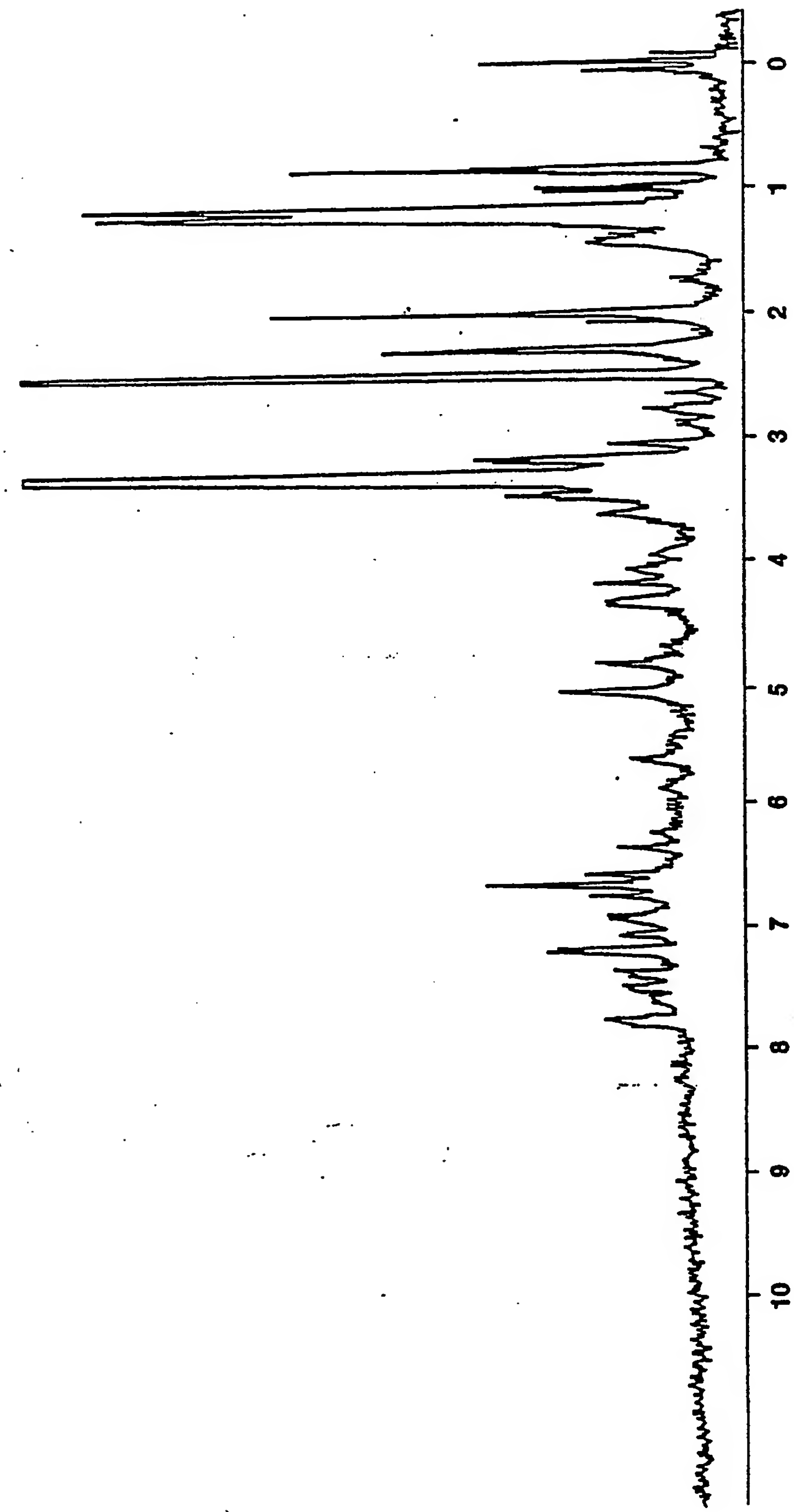
I.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PA

FIG. 8

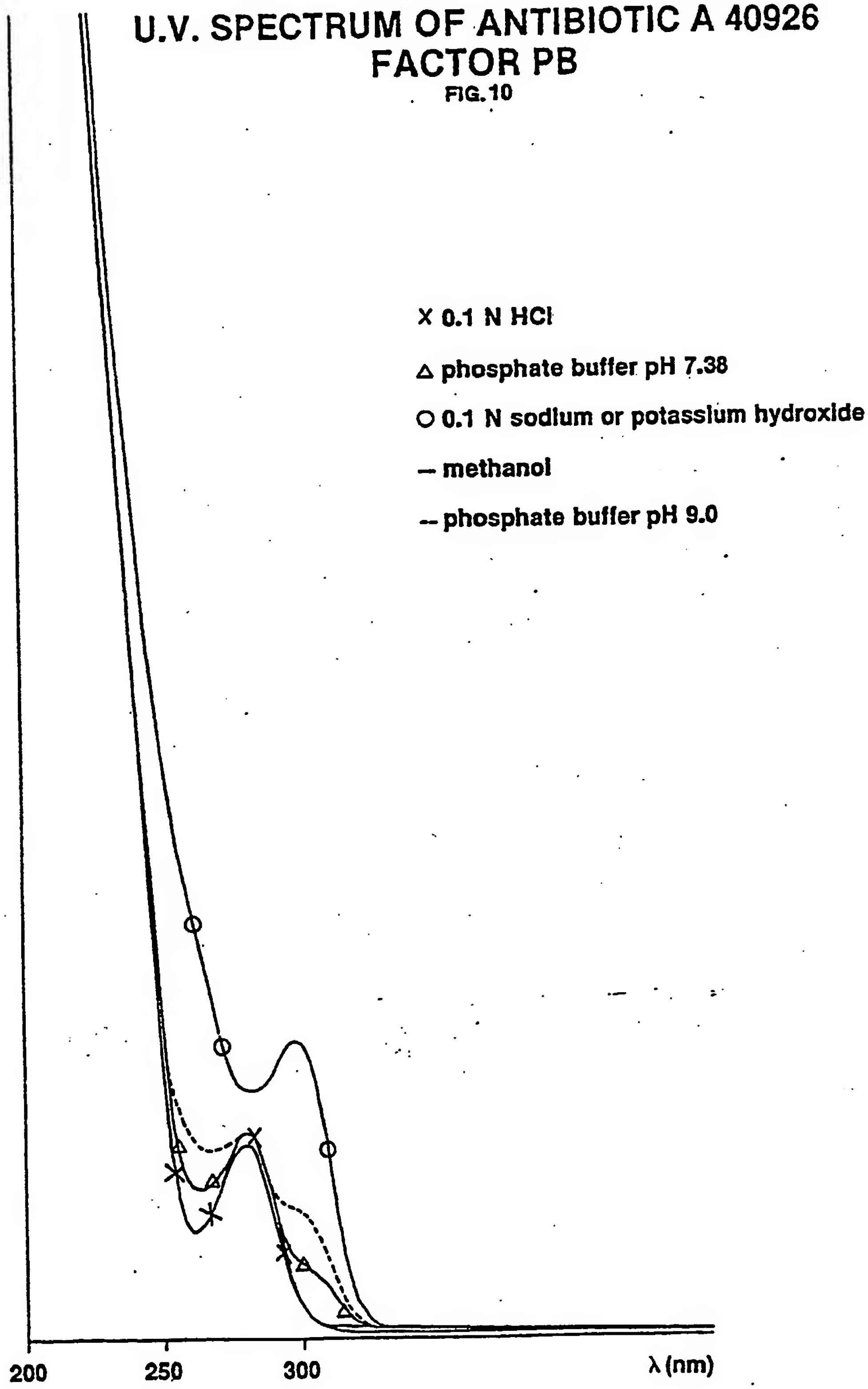


^{1}H.N.M.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PA

FIG. 9

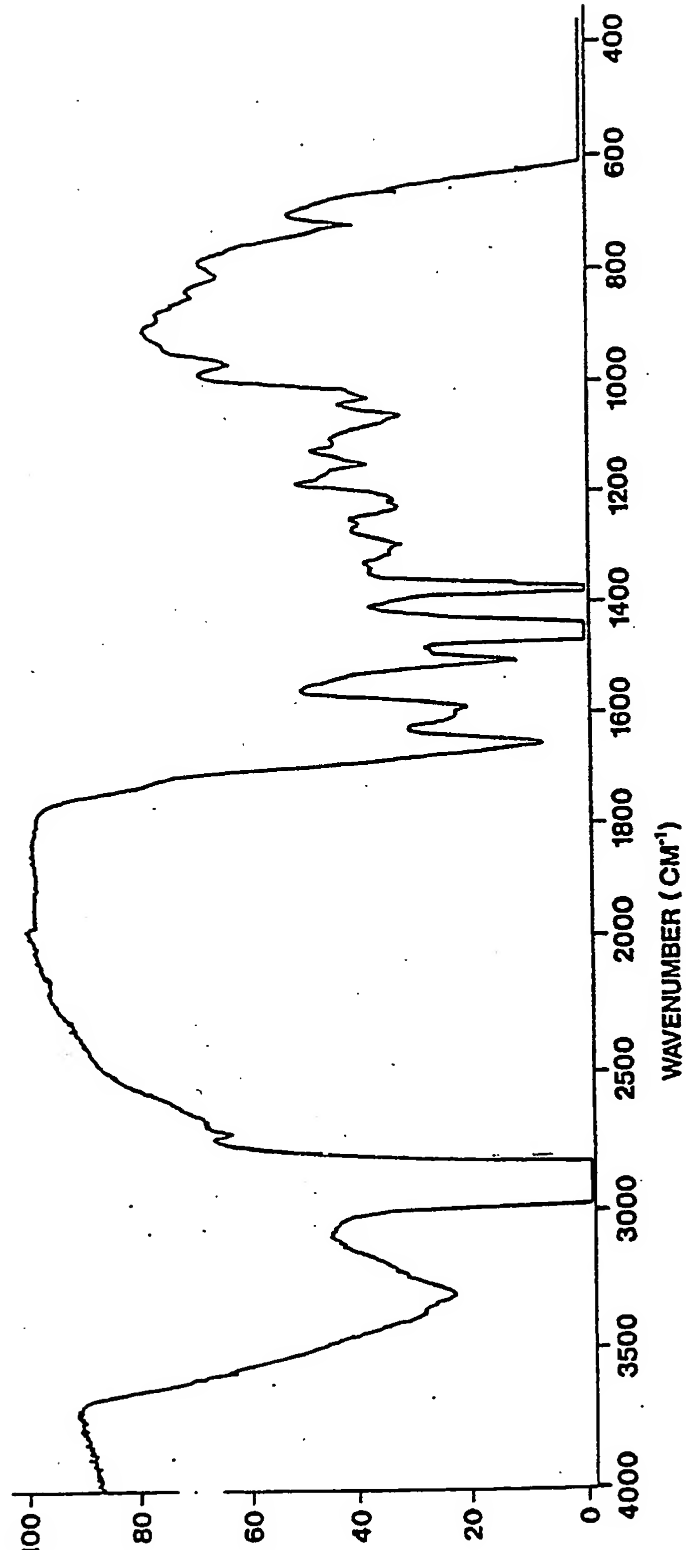


U.V. SPECTRUM OF ANTIBIOTIC A 40926
FACTOR PB
FIG.10



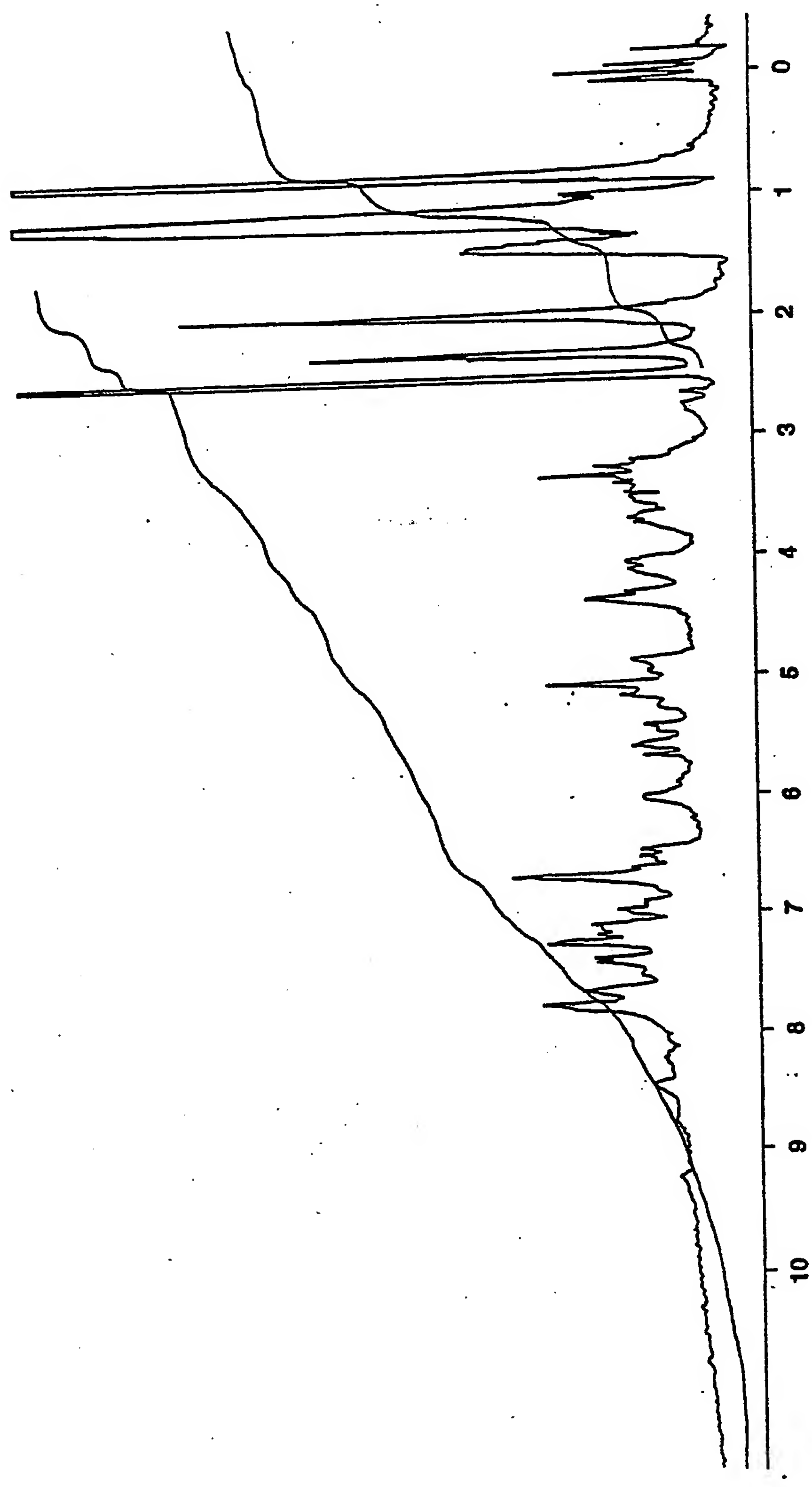
I.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PB

FIG. 11



^{1}H.N.M.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PB

FIG. 12



^{1}H.N.M.R. SPECTRUM OF ANTIBIOTIC A 40926 FACTOR PB

FIG. 13